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THE RELATION OF THE CHLORIDE METABOLISM TO THE TOXEMIA OF LOBAR PNEUMONIA*

PRELIMINARY REPORT

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LOBAR pneumonia is a complex disease in which any one of several factors may play the leading rôle. In some cases a bacteriemia may be the fatal feature, in others the deficient oxygenation may seem the most important factor. Quite typically, however, there is a toxemia which is responsible for many of the serious symptoms of the disease. The toxemia may perhaps arise indirectly from the bacteria concerned in the infection. It seems quite possible, however, that it may arise as a by-product of the bacterial infection which is the initial feature. This possibility is made more than probable by certain chemical findings which indicate a close relation of the toxemia of lobar pneumonia to other toxemias in which a bacterial factor can be excluded. The most typical chemically related toxemia is that occurring in intestinal obstruction.

The very low chloride excretion in acute lobar pneumonia has long been recognized as one of the most characteristic features of the disease. The behavior of the chlorides and the termination by crisis sharply differentiate lobar pneumonia from other acute pathologic conditions of the lung in which consolidation of tissue occurs. There is usually also increased protein destruction as indicated by the high nitrogen excretion.

In studying the chemical changes in the blood of the dog, following experimental intestinal obstruction, Haden and Orr[1] have found that chlorides disappear rapidly from the blood usually with a coincident alkalosis. These changes precede the tissue destruction and increased nonprotein nitrogen con-

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tent of the blood. The toxemia which is the most characteristic feature of high intestinal obstruction, does not arise until the chlorides have fallen below a certain level. Haden and Orr[2] have also shown that by keeping the blood chlorides near the normal level the toxemia may be prevented entirely. Dogs with obstruction of the jejunum have been kept alive as long as thirty-one days without developing a toxemia, while untreated dogs die in four days on an average. Likewise, supplying chlorides after the blood chlorides have reached a low level and the toxemia is already under way has usually influenced, most favorably, the course of the toxemia.

The fact that the toxemia does not develop in untreated animals until the chloride reserve is well depleted and can be prevented by providing an ample supply of chlorides, seems to prove that the utilization of chlorides is a protective mechanism on the part of the body. The changes are possibly explained by supposing that the toxic body arising as a result of the intestinal obstruction is converted by the sodium chloride into a nontoxic body with the formation of sodium bicarbonate as a by-product. Other possible explanations have been discussed elsewhere.[2]

The study reported here has been made with a view of determining, if possible, the relation of the chloride metabolism to the toxemia in lobar pneumonia as reflected in changes in the blood. The observations are rather fragmentary but seem worth while recording. Peabody[3] and others have studied the chlorides of the blood during lobar pneumonia and found them at a low level. It has been shown that the exudate in the consolidated lung contains only a small fraction of the chlorides retained. Analysis of other body tissues has not revealed any significant increase in chlorides. The very low excretion is due to the fact that the chlorides in the blood are below the renal threshold. No satisfactory explanation has been given for the disappearance of the chlorides from the blood. Peabody concludes that the chlorides retained may be generally distributed over the body.

Recent chemical studies on the blood in pneumonia[4] have shown also, that there is frequently a high nonprotein nitrogen in the blood. A large proportion of the nonprotein nitrogen is in the undetermined fraction, indicating that the changes observed are due to increased tissue destruction and not primarily to kidney retention. Studies on the CO_2 combining power of the blood plasma[5, 6, 7] show that there is seldom an acidosis, a condition so characteristic of other infectious diseases. There is often a true alkalosis. The low chloride excretion, the low level of blood chlorides, the frequent alkalosis, the increased protein destruction with the high level of nonprotein nitrogen in the blood in both lobar pneumonia and intestinal obstruction, indicate that there is a close relationship in the chemical changes taking place in the two diseases. As a further evidence of the similarity the occurrence of melanuria in both intestinal obstruction and in lobar penumonia has been observed.[8]

The very striking results in both preventing and combating the toxemia of intestinal obstruction by simply keeping the blood chlorides near the normal level, suggests its use in the chemically related toxemia of lobar pneumonia. None of the recent articles on the treatment of lobar pneumonia such as those in Nelson's Loose Leaf Medicine or the Oxford System

of Medicine mention the use of sodium chloride. Numerous articles were published twenty to twenty-five years ago on the saline treatment of lobar pneumonia. The first clinical report is that by Penrose in 1899.[9] Solis-Cohen[10] gives the credit for its use originally to F. P. Henry of Philadelphia who, he states, used it as early as 1890.

Most clinicians employed saline infusions as a means of producing increased urinary excretion. Others however definitely used sodium chloride as a detoxifying agent. Thus Sajous[11] thought that the early use of saline solution would produce the effects one tries to get with specific immune sera. Wakefield[12] pointed out that chloride is a sort of prototype of oxygen and can enter into combination in place of oxygen in conditions of suboxidation. This observer thought that antitoxins in pneumonia are produced by the catalytic action of oxidation. The utilization of chlorine, according to

TABLE I

CHEMICAL FINDINGS IN THE BLOOD OF PATIENTS WITH LOBAR PNEUMONIA

PATIENT	DAY OF DISEASE	BLOOD MG. PER 100 C.C. TOTAL NONPROTEIN NITROGEN	UREA NITROGEN	CREATININE	URIC ACID	CHLORIDES	CO_2 COMBINING POWER	URINE CHLORIDES	URINE NITROGEN	REMARKS
		mg.	mg.	mg.	mg.	mg.	Vol. per cent	per cent	per cent	
1	3	65.5	32.3	1.8	4.9	380	43.8	0.08	0.56	Crisis preceding night
	4	72.8	32.2	1.7	3.4	400	44.7	Faint trace	0.63	
	5	78.0	41.1	1.5	4.1	400	45.7			
	6	82.5	40.6	1.5	4.0	410	55.1			
	8	57.8	26.2	1.5	3.6	390	47.5	0.23	0.57	
	10	45.6	21.0	1.6	4.1	400	49.4	0.40		
	12	30.3	13.1	1.6	4.0	420	49.4	0.70	0.61	
2	3	30.0	14.0	1.6	4.4	360	47.5	0.10		
	4		15.8	1.7	3.9	350	46.6			
	6		14.0	1.7	3.6	370	49.4			
	8	29.4	12.1	1.8	4.7	360	49.4			
3	11	41.6	19.6	1.4	3.6	390	53.2	0.06		
	12	41.6	17.8	1.4	3.4	350	52.2	0.07		
	13							0.10	0.74	
	14	34.9	18.2	1.4	3.8	410	55.1			
	16	30.0	16.4	1.5	3.4	410	49.4	0.80		
4	6	45.0	16.3	1.5	3.6	400	37.6	Faint trace		
	7	60.0	28.5	1.4	3.6	430	45.7			Crisis
	8	69.0	31.8	1.8	4.6	430	41.9	0.05		
	9	57.8	27.0	1.2	5.3	440	45.7			
	10	63.2	21.4	1.2	4.6	410	43.8	0.05		
	11	54.6	16.4	1.2	4.7	470	45.7			
	12	56.4	16.8	1.2	4.7	460	46.6	0.08		
	13	42.8	14.5	1.2	4.8	470	36.2			
	15	32.6	15.4	1.3	4.7	460	44.7			
5	5	33.3	9.34	1.2	3.4	430	32.8	0.15		
	6	52.6	26.2	1.3	4.4	440	45.7	0.10		
6	7	98.4	50.0	1.8	3.6	390	36.2			

TABLE II

CHLORIDE BALANCE OF PATIENT WITH LOBAR PNEUMONIA TREATED WITH SODIUM CHLORIDE

DATE	SODIUM CHLORIDE GIVEN	URINE CHLORIDES		BLOOD	
		PER CENT	TOTAL	CHLORIDES (PER 100 C.C.)	CO_2 COMBINING POWER
Dec.	gm.		gm.	mg.	Vol. per cent
21	10	0.04	1.44		
22	16	0.07	1.54	370	
23	24	1.04	1.77	390	
24	24	0.08	2.48		
25	.0	0.15	9.30	370	51.3
26	.0	1.13	6.89	400	
27	.0	0.10	1.50	380	
28	.0	0.30	7.50		
29	.0	0.40	5.60	480	55.1
30	6	0.40	5.60	480	
31	6	0.41	4.10	380	
Jan.					
1	6	0.60	5.04	400	46.6
2	6	0.74	6.60		
3	6	0.87	5.22		
4	6	0.80	6.00		
5	6	0.73	1.18		
6	6	1.18	3.78		
7	6	1.23	8.00		
8	6	1.00	11.50		
9	6	1.12	6.77		
10	6	1.00	7.00		
11	6	1.00	7.00		
Total	152		112.61		

this view, is a protective measure on the part of the body in an attempt to hasten the formation of antibodies. Peabody,[3] in studying the blood chlorides in pneumonia, gave small amounts of salt to note what effect it would have on the blood chlorides. Prigge[13] has reported eleven cases of pneumonia in which a single intravenous injection of 20 to 38 gm. of sodium chloride was given. Every one of the patients showed a rise in blood pressure following the injection and all recovered. Prigge states that all of the chloride was excreted within four days after it was given, and that the chloride metabolism in pneumonia patients differed from normal controls only in the greater length of time required to excrete the chloride injected.

It is a surprising but well proved fact that dogs with high intestinal obstruction die more quickly when given a plentiful supply of distilled water than if untreated.[2] Considering the close chemical relationship of the two conditions it seems a question whether it is wise in lobar pneumonia to force water and other fluids containing very little salt.

The observations reported here are based on the study of seven cases during an acute lobar pneumonia. In four, repeated complete chemical analyses of the blood have been made over a period of several days; in two, only a few analyses were made; and in one, only the chlorides and carbon dioxide combining power were studied. The analyses in six cases are shown in Table I. The results are quite variable. In all, the blood chlorides are below the normal level. In five, the nonprotein nitrogen and urea nitrogen are above the upper limit of normal. Two of the patients died. In the other

three the nonprotein nitrogen and urea nitrogen returned to normal as the blood chlorides reached the normal level. The sixth case showed a low blood chloride without a rise in nitrogen. The urine chlorides were estimated in five cases and found very low in all.

The chloride metabolism of one patient treated with sodium chloride is shown in Table II. This patient was a young robust man admitted to the hospital with an acute appendiceal abscess. A typical lobar pneumonia developed the day following operation. At the outset only the right lower lobe was consolidated. Later the middle and upper lobes were similarly involved. The blood pressure remained normal throughout the illness. One other patient with lobar pneumonia due to hemolytic Streptococcus responded equally well to treatment. This patient developed the lung infection following a cholecystectomy. The chart of the chloride metabolism is of little value, however, since some of the chloride tablets were passed unchanged in the stools.

The patient shown in Table II was given 74 gm. of sodium chloride by mouth in the first four days of treatment besides that taken in with food. During this period the total excretion in the urine was 7.23 gm. and the blood chlorides remained well below the normal level. A total of 152 gm. of salt was given by mouth during the period of observation. For the greater part of this time he was taking a soft diet which included about 5 gm. of salt per twenty-four hours. The total excretion was 112.6 gm. This patient had no acidosis.

DISCUSSION

The cases reported here showed no significant decrease in the carbon dioxide combining power emphasizing again the absence of an acidosis in lobar pneumonia. The creatinine is unchanged and the uric acid is little above the upper limit of normal. The low chlorides and increase in nonprotein nitrogen and urea nitrogen are the significant findings. The undetermined fraction of the nonprotein nitrogen is quite high. These findings suggest that the changes observed are due to increased protein destruction instead of kidney retention. It is most probable that the accelerated protein metabolism is generalized and that the products responsible for the toxemia come from body protein largely independent of direct bacterial action.

The urine chlorides are very low, and the urine nitrogen quite high. The urine chlorides give little clue to the level of chlorides in the blood and body tissues.

The chloride metabolism of the patient treated is very striking. He was given 152 gm. of sodium chloride by mouth in addition to that taken in with food. The latter is estimated as being about 5 gm. per day. Only 112 gm. of the 152 gm. given is accounted for in the urine in twenty-one days. The very large amount of salt which will be absorbed in toxemia with chloride depletion is significant. The findings here are very similar to those in intestinal obstruction. One patient with intestinal obstruction has been observed to absorb 90 gm. of sodium chloride in thirty-six hours when given by hypodermoclysis, and excrete during the same time only 1.8 gm.[14]

The clinical condition of the patient treated was very satisfactory, even when one entire side was consolidated. The blood pressure remained normal. One of the most serious aspects of pneumonia is the low blood pressure. The chlorides must play an important part by osmotic action in maintaining the pressure within the vessels.

Sodium chloride was given in 1 gm. enteric coated tablets (Hynson, Westcott, and Dunning). The administration of sodium chloride in this way is a simple procedure. It is suggested that such use is indicated in all cases of lobar pneumonia in which there is a low level of chloride excretion without an evident kidney lesion.

The results in a group of patients so treated will be reported later.

SUMMARY

The blood chemical analyses of a series of patients with lobar pneumonia are reported. The blood chlorides are low, the nonprotein and urea nitrogen are high; there is no acidosis. The changes observed are probably due to a generalized protein destruction. Supplying the missing chloride seemed to influence markedly the course of the disease. In all cases of lobar pneumonia sufficient chloride should be given to keep the blood chlorides at or near the normal level.

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STUDIES ON THE PHYSICAL AND COLLOIDAL CHEMISTRY OF ARSPHENAMINE

PART I. THE DETERMINATION OF AN ISOELECTRIC POINT*

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ALTHOUGH years have passed since salvarsan was first presented to the world by Ehrlich and his collaborators, but little real information has been acquired as to its chemical action when in solution, the cause of its frequent abnormal toxicity, and the complex problem which its physiologic activity in the body presents. In spite of the vast amount of work that has been done upon this drug, from a chemical as well as a clinical viewpoint, it is impossible to explain the apparent differences which occur in the different commercial arsphenamines on the market today. There is lacking the reason for their variations in color, arsenic content, solubility, toxicity and therapeutic activity.

The application of pure physical or colloidal chemistry to these investigations has thus far been scanty. Bauer (1919) in his study of the colloidal properties of arsenicals of the arseno type measured their diffusibility by means of the ultra filter according to the method of Bechold (1919). He found that the greater part of the salvarsan hydrochloride in solution was retained by the filter, thus showing the presence of colloidal properties. In addition, he noted that a freshly alkalinized solution diffused more slowly than did a solution made from the dry commercial salvarsan sodium.

Sherndal (1921) showed the colloidal nature of arsphenamine very plainly He found that the conversion of the "base" into the dihydrochloride in an ionized medium gave, in every case, a product whose gelatinous characteristics, as evidenced by the viscosity of the aqueous solutions and its insolubility in methyl alcohol, were very much more marked than when the material was prepared in a nonionizing medium. He was able to change a difficultly soluble preparation into a readily soluble one and vice versa.

Raiziss and Gavron (1922) made a qualitative study of the diffusibility of several arseno preparations through parchment membranes. Both the disodium salts and the dihydrochlorides possessed colloidal properties as shown by their partial retention. The sodium salt of arsphenamine diffused to a greater extent than the dihydrochloride.

Z. Klemensiewicz (1920) studied the viscosity of aqueous salvarsan solutions. He found that upon standing, the viscosity of the solution arose to a

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maximum, the rate being proportional to the concentration. He classified aqueous "606" solutions as emulsoids together with gelatine, albumin, etc. He suggested the idea that solutions of salvarsan give rise to strong polymerization of the molecule, probably accompanied by hydration.

Jean Oliver (1923) stated that arsphenamine combined with the red blood cells and, in the presence of electrolytes, coagulation resulted. This coagulation, he claimed, was inhibited by the addition of hydrophylic colloids, such as gelatine, gum arabic, starch, etc., which by their combination with the arsphenamine prevented its union with the blood cells and the electrolyte, thereby preventing coagulation. The colloid played the rôle of a protective colloid.

In contrast, however, Freundlich, Stern and Zoeher (1923) stated that in the presence of gelatine, arsphenamine was sensitized toward the coagulating effect of electrolytes. In hydrochloride solutions as well as in the alkalinized solutions a .4 molal concentration of NaCl caused marked clouding. Freundlich remarked that this fact should be emphasized. "It should not be overlooked that the hydrophyl solutions so often designated as protective colloids, as gelatine for example, very often exert a sensitizing effect which is exactly opposite. We would put the solutions of salvarsan and neosalvarsan in the class of semicolloids, particularly like the colloidal electrolytes, of which class soap solutions are more familiar. As contrasted with these there is present in salvarsan a colloidal cation but no colloidal anion as in neosalvarsan and the other materials mentioned."

Voegtlin, Johnson and Dyer (1924) in their work on the viscosity and toxicity of arsphenamine solutions showed that a given viscosity of a hydrochloride solution apparently corresponded to a definite toxicity of the product, regardless of the method of manufacture. Also, the higher the viscosity of the hydrochloride, the higher its toxicity. Yet, the hydrochloride solutions which had the highest toxicity showed the lowest toxicity when converted into the sodium salt.

It now remains to establish something definite regarding the physical state and the colloidal properties of this drug. From its accepted chemical structure as well as from its salt-forming properties it is obviously amphoteric, i.e., it can act either as an acid or as a base. The important thing in the study of an amphoteric material is the position of its isoelectric point.

The conception of an isoelectric point as applied to proteins was due to W. B. Hardy (1900) when he noticed that egg albumen when put into an electrical field migrated in an opposite direction according to whether the reaction of the fluid was acid or alkaline. When the solution was neutral cataphoresis was negligible and under such a condition the particles must be isoelectric. In 1905, Hardy in his paper on "Colloidal Solution" recognized the amphoteric nature of proteins and stated that they could react with either an acid or a base. When the methods of measurement of the hydrogen-ion concentration were developed by H. Friedenthal and Sorensen it became possible to determine the isoelectric point of such ampholytes. Michaelis in 1910 used the method of Hardy to study the migration of albumin particles in an electrical field and according to him, the isoelectric point is that hydrogen-ion

concentration at which the particles migrate neither to the anode or the cathode. Loeb (1919) showed that ash-free proteins could be prepared by washing the protein at its isoelectric point, since there it combined with a minimum of anions or cations. His procedure was to determine the P_H of a protein solution potentiometrically and then add acid or alkali sufficient to bring it to its isoelectric point.

Other methods have been developed for the determination of isoelectric points which are more convenient than cataphoresis measurements. They are based on the fact that at the isoelectric point the osmotic pressure, the viscosity, alcohol required for precipitation, the conductivity, the swelling are at a minimum. Levene and Simms (1923) in their paper, "Calculation of Isoelectric Points" conceive of the isoelectric point of an amphoteric substance as that hydrogen-ion concentration at which it is ionized equally as an acid and as a base.

They show that the usual formula for single ampholytes:

$$\text{I.P.} = \sqrt{\frac{K_a}{K_b} K_w}$$

may be used to calculate the approximate isoelectric point of polyacidic, polybasic ampholytes by using the K_a and K_b of the strongest acid and strongest basic group.

Now, if it should be desired to study the colloidal properties of arsphenamine it would be necessary to first obtain the material free of electrolytes or else the experimental results would mean little. Chemically pure arsphenamine has never been made. If a protein can be purified by washing at its isoelectric point a similar treatment might be effective with arsphenamine. It was deemed fundamental then to determine the isoelectric point so that there might be a foundation upon which future physical investigation might rest.

Conductivity measurements on arsphenamine solutions seemed to offer the most applicable method of attack. It was recognized that due to the partial insolubility of the drug at certain hydrogen-ion concentrations and because of the possibility of polymerization and hydration, that a minimum conductivity measurement might or might not be the exact criteria of the position of the isoelectric point. However, it was hoped that conductivity measurements might shed some light upon the physical condition of arsphenamine in solution. The purpose of this investigation, then, was to establish the isoelectric point of arsphenamine as indicated by a minimum conductivity of the solution and to determine the alkalinization curve of arsphenamine in terms of P_H values.

METHODS EMPLOYED

Conductivity Measurements.—The new feature of the conductivity system was the source of alternating current. A radiotron audion bulb was used to generate pure sign wave alternating current. The authors are indebted to Geo. C. Crom of the Engineering Dept. of the DeForest Radio Telephone and Telegraph Co., through Dr. DeForest, for the following set-up which is not only

simple in its construction but also decidedly effective for this sort of work. (See diagram.)

The three "Duo-Lateral" honeycomb coils of the indicated number of turns were seated in a regular frame. The three condensers had a capacity of 0.1, 0.1 and 1.0 microfarads respectively. A radiotron tube U.V. 201 was used. The entire circuit was housed in a cabinet according to the most improved radio methods. Ninety volts were impressed on the plate by the "B" battery and a six volt storage battery served as the "A" battery.

Not enough can be said of the efficiency of this instrument as a generator of alternating current, (DeForest 1920), for conductivity work. The aggravating hum of a buzzer is absent. The cost of a Vreeland oscillator often makes its use prohibitive. This instrument is relatively quiet, enough so that it can be kept within easy reach of the rest of the apparatus and the constant temperature bath. Simply by turning the knob of the rheostat which controls the current passing through the filament, a smooth, clear tone is heard in the phones. This can be regulated at will to any intensity required by the operator. In addition, the current flows through the cell for a minimum length

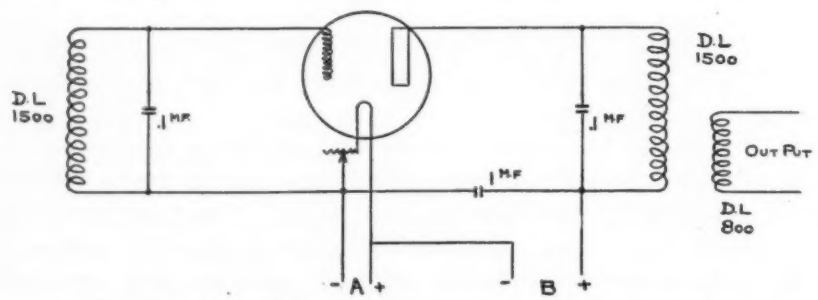


Diagram of Vacuum Tube as a Generator of Alternating Current.

of time and this of course is essential with arsenic-containing solutions, lest the platinum be poisoned. If the bridge drum is perfectly clean and preferably when the wire is covered with a slight film of oil, an *absolute* and *reproducible* null point can be readily obtained between two or three graduations on the drum scale. It was not necessary to have the current passing through the cell for more than half a minute at a time in order to make a measurement and by way of precaution the current was always shut off before the readings were recorded. Three different readings were taken and their mean calculated value was accepted as the conductance of the solution.

The cells were calibrated according to the weight method of Kraus and Parker (1922) using 0.1 N KCl as the standard. The conductivity water which was used in all solutions, was made by redistilling freshly distilled water from alkaline permanganate and calcium hydroxide through a block tin condenser and collecting in Jena glass bottles. When used, the water protected by soda lime, was withdrawn through a Pyrex siphon which had a ground-in stopper at the lower end.

Special cells were made of Pyrex in which the platinum disks 1.0 cm. in diameter were in a perpendicular position about 2.0 cm. apart. A small tube

passed down through the ground-in glass stopper so that an inert gas could be passed over the solutions in order to prevent atmospheric oxidation. An exit tube was placed near the top of the stopper.

The cell constants were calculated according to the usual formula:

$$K = C \times R \quad \begin{matrix} C = 0.0128988 \text{ for } 0.1 \text{ KCl } (25^\circ \text{ C.}) \\ R = \text{resistance of solution.} \end{matrix}$$

The cell constants calculated out as follows:

Cell	C.	R.	K.
A.	0.0128988	14.043	0.181137
B.	"	19.069	0.245950
C.	"	14.925	0.192500

Parallel cells were used for each measurement and the mean of their calculated specific conductivities was taken as the value for the solution. In each series a third cell was brought into use on one or more solutions in order to check the accuracy of the parallel cells and to be sure that they were functioning correctly.

When this investigation was undertaken it was feared that, due to the presence of the arsenic in the solutions, the platinum electrodes might be poisoned and caused to hinder correct measurements. Although some trouble did occur it was only temporary. Great care had been taken that the current flow through the cells for a minimum length of time. Also, when not in use the cells were kept filled with conductivity water. In spite of this there seemed to be a slight absorption on the platinum black as shown by the yellowness of the water in the cells after they had been in constant use for a day or so. The first indications of trouble came after the cells had been in use for about four months. There was an irregularity of sound in the phones. A slight "putt, putt," could be heard and often a ringing sound

TABLE I

CONDUCTIVITY MEASUREMENTS ON ARSPHENAMINE E

NO.	C.C. 0.1 N NaOH PER 0.1 GM.	CELL	L.	MEAN L.	DIFFERENCE
149	0.0	A	0.003171		
		C	0.002993	0.003082	0.000178
151	1.0	A	0.002416		
		C	0.002556	0.002486	0.000140
152	2.0	A	0.002201		
		C	0.002169	0.002185	0.000032
153	2.6	A	0.002132		
		C	0.002038	0.002085	0.000094
154	2.8	A	0.002106		
		C	0.001954	0.002030	0.000152
155	3.0	A	0.002159		
		C	0.000159	0.002159	0.000000
157	5.0	A	0.002664		
		C	0.002585	0.002624	0.000079
150	8.5	A	0.004302		
		C	0.004186	0.004244	0.000116

All solutions were made up on the basis of 1.0 gm. sample of arsphenamine made up to 200 c.c. in a graduated flask. Column 2 gives the c.c. 0.1N NaOH added per .1 gm. sample. Column 6 shows the difference between the two cells to be small.

would predominate. The strange thing about it was that after two or three fresh solutions had been used in the cell it would again function correctly. When in their worst condition readings on the drum meant nothing as a null point was found to be practically constant, regardless of the resistance out. No readings were taken under such conditions.

Finally, the now greyish platinum black was dissolved off with hot aqua regia (the solution being disregarded) and the cells were filled with hot acid chromate for a day. Then after a thorough electrolytic cleansing, they were replated and once more behaved in a normal manner. Any ringing sound usually indicated trouble which always resulted in the necessity of having to repeat the measurement.

The accuracy of the conductivity work is best attested by a typical table (Table I).

Hydrogen-ion Concentration Measurements.—Hydrogen-ion concentration measurements were made in parallel on each solution and simultaneous with the conductivity measurements. Clark shaking electrode vessels were used and the electrodes were covered with platinum black just sufficient to conceal the metal surface and make them jet black and glossy. A saturated calomel cell was made up according to the method of Fales and Vosburg (1918) and a saturated KCl chain was used. The apparatus was kept in an air bath at 25 ± 0.1° C. Electrolytic hydrogen was supplied by a small generator employing platinum electrodes and a slight evolution of gas was maintained continuously when the apparatus was not in use. Constant pressure in the two cells was established by the use of a water manometer within the air bath. Barometric pressure corrections were neglected. The readings were made on a Leeds and Northrup type K potentiometer, and the galvanometer possessed a sensitivity of 0.025 microamperes per millimeter.

Calculations for P_H values were made according to the usual formula:

$$P_H = \frac{\text{Obs.E.M.F.} - \text{Cal}^*}{0.0001983\ T}$$

Due to the slowness with which the precipitate containing solutions came to equilibrium with the hydrogen, twenty minutes was taken as the time of shaking for all solutions before making a final reading. A preliminary reading was made on each solution after shaking for 15 minutes and then a fresh aliquot was placed in the vessels and they were shaken for twenty minutes more. Thus the final and accepted reading represents the condition of the solution after a thirty minute interval. This preliminary measurement was found to be very helpful as it indicated whether or not the parallel electrodes were acting together and correctly. An index of the accuracy of the P_H measurements may be found in Table II.

EXPERIMENTAL WORK

Acknowledgment is made to the following manufacturers for their cooperation in supplying the material used in this investigation: Powers, Weightman, and Rosengarten; The Dermatological Research Laboratories,

*E. M. F. Cal, because of consistent reading was taken as 0.2478 volts.

TABLE II

P MEASUREMENTS ON ARSPHENAMINE E

EXP.	C.C. 0.1 N NaOH PER 0.1 GM.	TIME OF SHAKING	ELECTRODE A.	ELECTRODE B.	RECORDED VALUE	P_H
149	0.0	15	0.3889	0.3889		
		20	0.3892	0.3900	0.3896	2.390
151	1.0	15	0.3922	0.3924		
		20	0.4052	0.4054	0.4053	2.664
152	2.0	15	0.4167	0.4162		
		20	0.4166	0.4166	0.4166	2.855
153	2.6	15	0.4449	0.4459		
		20	0.4456	0.4462	0.4459	3.352
154	2.8	15	0.4496	0.4497		
		20	0.4492	0.4488	0.4490	3.403
155	3.0	15	0.4547	0.4549		
		20	0.4546	0.4544	0.4545	3.500
157	5.0	15	0.8484	0.8463		
		20	0.8421	0.8423	0.8422	10.05
158	7.0	15	0.9174	0.9164		
		20	0.9134	0.9110	0.9122	11.24
150	8.5	15	0.9321	0.9332		
		20	0.9288	0.9284	0.9286	11.52

Column 3 gives the time of shaking in minutes; column 4, the voltage readings of the parallel hydrogen electrodes; column 5, the equivalent PH values.

Philadelphia, Pa.; E. R. Squibb and Sons, New Brunswick, N. J.; Mallinckrodt Chemical Works, St. Louis, Mo.; Kober Chemical Co., Inc., Hastings-on-Hudson, N. Y.; and H. A. Metz Laboratories, Inc., New York, N. Y. In addition, two types of laboratory arsphenamine and two types of salvarsan sodium were made by the authors.

Laboratory Preparations. Arsphenamine D.—Sixty gm. moist "base" was dissolved in 360 c.c. absolute methyl alcohol by the addition of 6.5 gm. 100 per cent HCl in methyl alcoholic solution. The resulting dihydrochloride solution was filtered and precipitated from five liters of absolute ether. The precipitated material, after having been collected on a filter in an inert atmosphere was washed with ether and then dried in vacuo over sulphuric acid. At the end of ten days the arsenic content was 31.95 per cent (control experiment).

Arsphenamine E.—Sixty gm. of the same moist "base" as used in arsphenamine D was suspended in ten liters of distilled water and kept covered with an inert atmosphere. A series of filtercones were inserted into the suspension and while the solution was vigorously stirred, suction was applied. As fast as the water was removed distilled water having a P_H of about 4.0 was added until thirty liters of this acidified water had passed through the suspended "base." Following this, ten liters having a P_H of 3.4 were sucked out. The "base" was then filtered off and made into the dihydrochloride as with D. The arsenic content of this preparation after ten days was 32.35 per cent.

The reason for this modification was to ascertain the effect of washing arsphenamine at its isoelectric point. No marked difference was detected in the physical studies made upon the two products. Toxicity tests have not been completed.

The technic used in preparing and handling the solutions is very im-

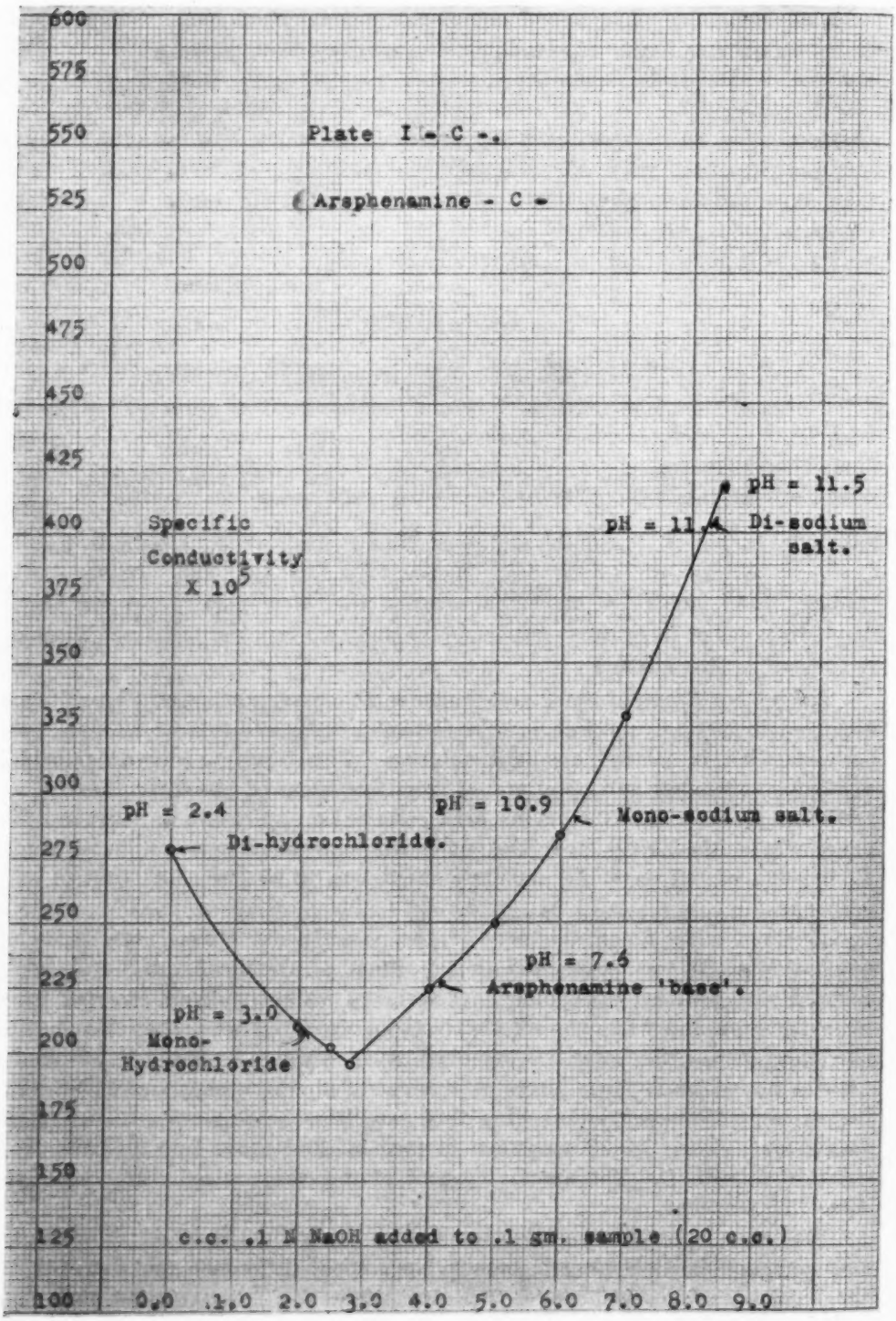


Plate I - C -.
Arsphenamine - C -
Specific Conductivity X 10^5
pH = 11.5
pH = 11.4
Di-sodium salt.
pH = 10.9
Mono-sodium salt.
pH = 2.4
Di-hydrochloride.
pH = 7.6
Arsphenamine 'base'.
pH = 3.0
Mono-Hydrochloride
c.c. .1 N NaOH added to .1 gm. sample (20 c.c.)
600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0

portant as a time factor must be considered. In all cases a uniform concentration of 1 gm. sample made up to 200 c.c. (approximately 0.01 molal) was used. A series of solutions were prepared which contained increasing amounts of sodium hydroxide up to 8.5 c.c. 0.1 N NaOH per 0.1 gm. sample, which corresponds to a correctly alkalinized solution (Public Health Reports, 1922). It is imperative that the sample be completely in solution before the addition of alkali. The alkali was added to the solution from a standard burette and the graduated flask was rotated gently during such an addition in order to prevent undue clumping of any formed precipitate.

When the solution was homogeneous the hydrogen electrode vessels were filled. Following this, some of the solution was placed in the conductivity cells and the time was noted. At the end of fifteen minutes the preliminary potentiometric reading was taken and a second aliquot placed in the vessels. When twenty minutes had passed, the conductivity measurements were made, the cells rinsed out and filled with conductivity water. Then at the end of the second period the final P_H reading was taken. The best proof of the uniformity of this technic came in the fact that the points shown on the curves were not made in consecutive order but that it was possible to reproduce the measurements and insert unknown points in the curves at any time and have them fall within experimental error. All measurements were made at 25° C.

TABLE V

ARSPHENAMINE C

EXP.	C.C. 0.1 N NaOH ADDED PER 0.1 GM.	SP. C. × 10^4	VOLTS	P_H
244	0.0	27.82	0.3908	2.419
246	2.0	21.05	0.4251	3.000
248	2.5	20.21	0.4358	3.180
247	2.8	19.49	0.4485	3.395
250	3.5	21.84	0.5184	4.578
251	4.0	22.43	0.7098	7.816
252	5.0	24.93	0.8531	10.24
249	6.0	28.38	0.8970	10.98
253	7.0	32.96	0.9169	11.32
245	8.5	41.78	0.9288	11.52

TABLE XV

SPECIFIC CONDUCTIVITY INCREASE UPON ALKALINIZATION

ARSPHENAMINE	DIHYDROCHLORIDE	DISODIUM SALT	INCREASE
A	28.46 × 10^4	41.65 × 10^4	13.19 × 10^4
B	28.43	38.82	10.39
C	27.82	41.78	13.96
D	29.97	42.41	12.30
E	30.82	42.44	11.62
F	27.01	41.03	14.02
G	23.20	41.24	18.04
H	27.19	39.93	12.74
I	26.88	41.96	15.08

Four types of curves were plotted from the data obtained in each series. These were: Type I, specific conductivity versus c.c. 0.1 N NaOH added per 0.1 gm. sample; type II, specific conductivity verses P_H values of the solutions; type III, specific conductivity versus NaOH required for alkalinization and

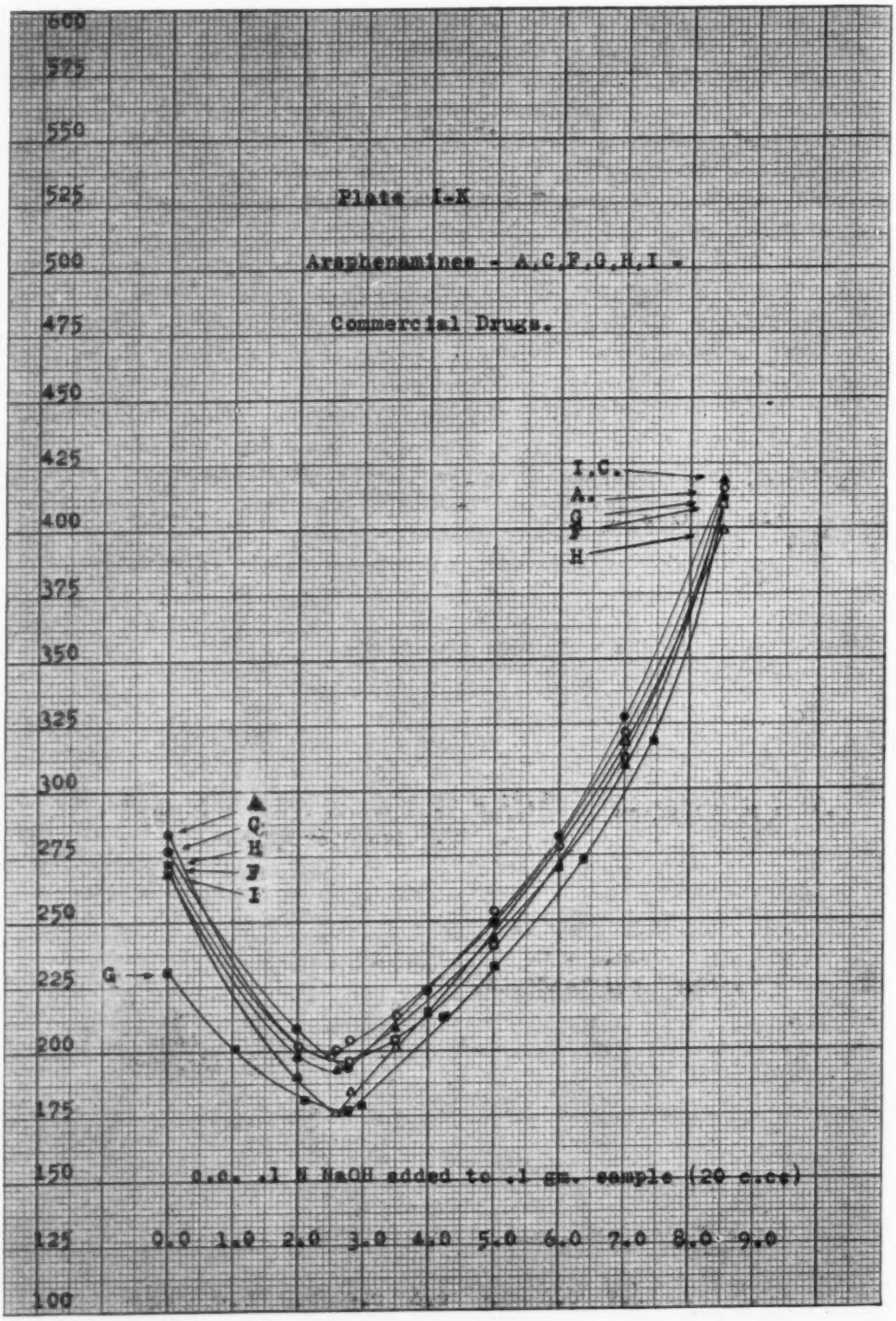


Plate I-K
Arsphenamines - A.C.F.G.H.I -
Commercial Drugs.
I.C.
A.
G
F
H
C
H
F
I
G
c.c. .1 N NaOH added to .1 gm. sample (20 c.cs)
0.0 1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0
600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100

HCl added to convert the formed disodium salt back into the dihydrochloride; type IV, P_H values of solutions versus alkali and acid added as in III. The curves of types III and IV will be discussed in a later paper.

Table V gives the data of a typical series of experiments.

Plate I-C illustrates the curves of type I in showing that upon the addition of NaOH to the solution of arsphenamine dihydrochloride the specific conductivity drops to a minimum after about 2.8 c.c. 0.1 N NaOH have been added per 0.1 gm. sample. A further addition of alkali caused an increase in the specific conductivity of the solution. This was characteristic of all of the arsphenamines examined. Plate I-K indicates the phenomena as presented by the six different commercial arsphenamines used in this investigation.

The increase in specific conductivity of the respective arsphenamines upon alkalinization is shown by Table XV. It is to be noted that a marked difference occurred only in the case of arsphenamines B and G. Arsphenamine B was studied for comparative purposes because it showed visible signs of some sort of decomposition. The drug instead of being bright yellow had a noticeable brownish cast and its solution had a brownish tinge. Table XVI shows again where this material differed slightly from the other materials in that the P_H of its alkalinized solution was a bit lower. Arsphenamine G showed no signs of decomposition. It was characterized by exceptional solubility for arsphenamine. Its solution was clear and citron yellow. Since arsphenamines D and E represent the regular and the washed laboratory preparations it was thought possible that a difference might show up at this point. Such, however, was not the case.

Plate II-C shows the variation of the P_H of the solution with the specific conductivity during alkalinization. This type of curve is important because the P_H at the minimum indicated represents the isoelectric point of arsphenamine. Plate II-K gives the analogous curves for the six commercial arsphenamines studied. These appear to fall in two classes: Arsphenamines C, D and H which show a sharp break at the isoelectric point, the minimum, and arsphenamines A, E, F, and G wherein the slope of ascent is more gradual.

Table XVI shows the change of the P_H of the respective solutions during the alkalinization.

TABLE XVI

COMPARISON OF PH CHANGES
(Alkalinization)

SAMPLE	C.C. 0.1 N NaOH ADDED										C.C. 0.1 N NaOH AT MINIMUM SP. C.	I. P.
	0	1	2	3	4	5	6	7	8	8.5		
A	2.41	2.62	3.01	3.84	8.55	10.16	10.74	11.22	11.43	11.55	2.5	3.25
B	2.36	2.76	2.96	3.75	6.90	9.62	10.45	10.96	11.25	11.40	2.8	3.42
C	2.42	2.68	3.00	3.70	7.80	10.24	10.98	11.32½	11.50	11.52	2.8	3.39
D	2.40	2.63	2.81	3.58	6.40	10.14	10.68	11.10	11.40	11.56	2.8	3.41
E	2.39	2.66	2.85	3.20	6.24	10.05	10.76	11.24	11.45	11.52	2.8	3.40
F	2.43	2.62	3.06	3.65	6.67	10.14	10.70	11.00	11.36	11.51	2.8	3.39
G	2.48	2.75	3.15	4.06	9.32	10.09	10.40	10.75	11.20	11.42	2.8	3.40
H	2.35	2.58	2.96	3.85	8.46	10.32	10.88	11.24	11.45	11.54	2.6	3.40
I	2.41	2.52	2.95	3.50	6.97	10.24	10.81	11.18	11.42	11.56	2.6	3.34
J	2.41				(8.53—A, C, G, H)					11.40		
	2.47				(6.63—B, D, E, F, I)					11.27		

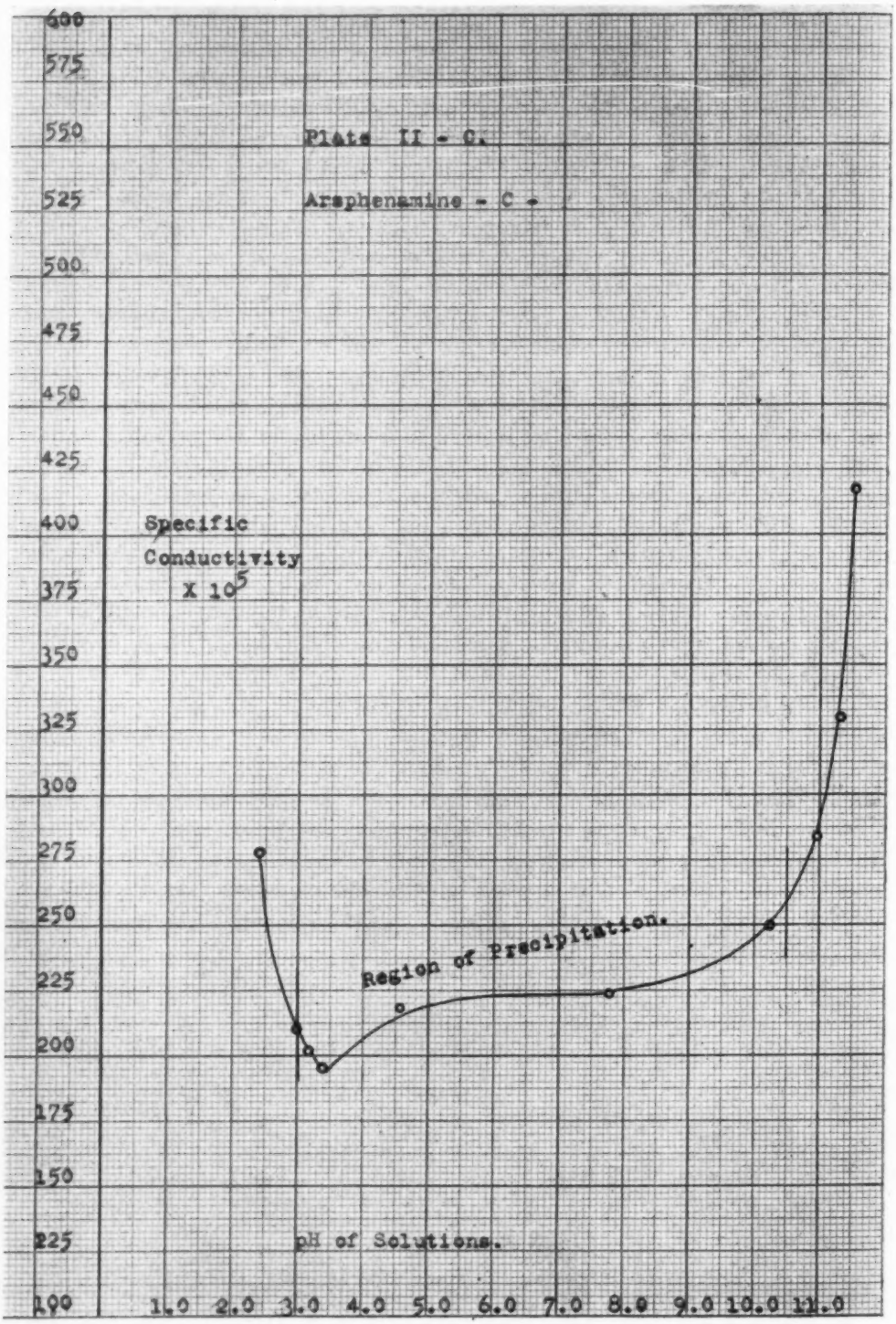


Plate II - C.
Arsphenamine - C +
Specific
Conductivity
X 10^5
Region of Precipitation.
pH of Solutions.
600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
225
100
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0

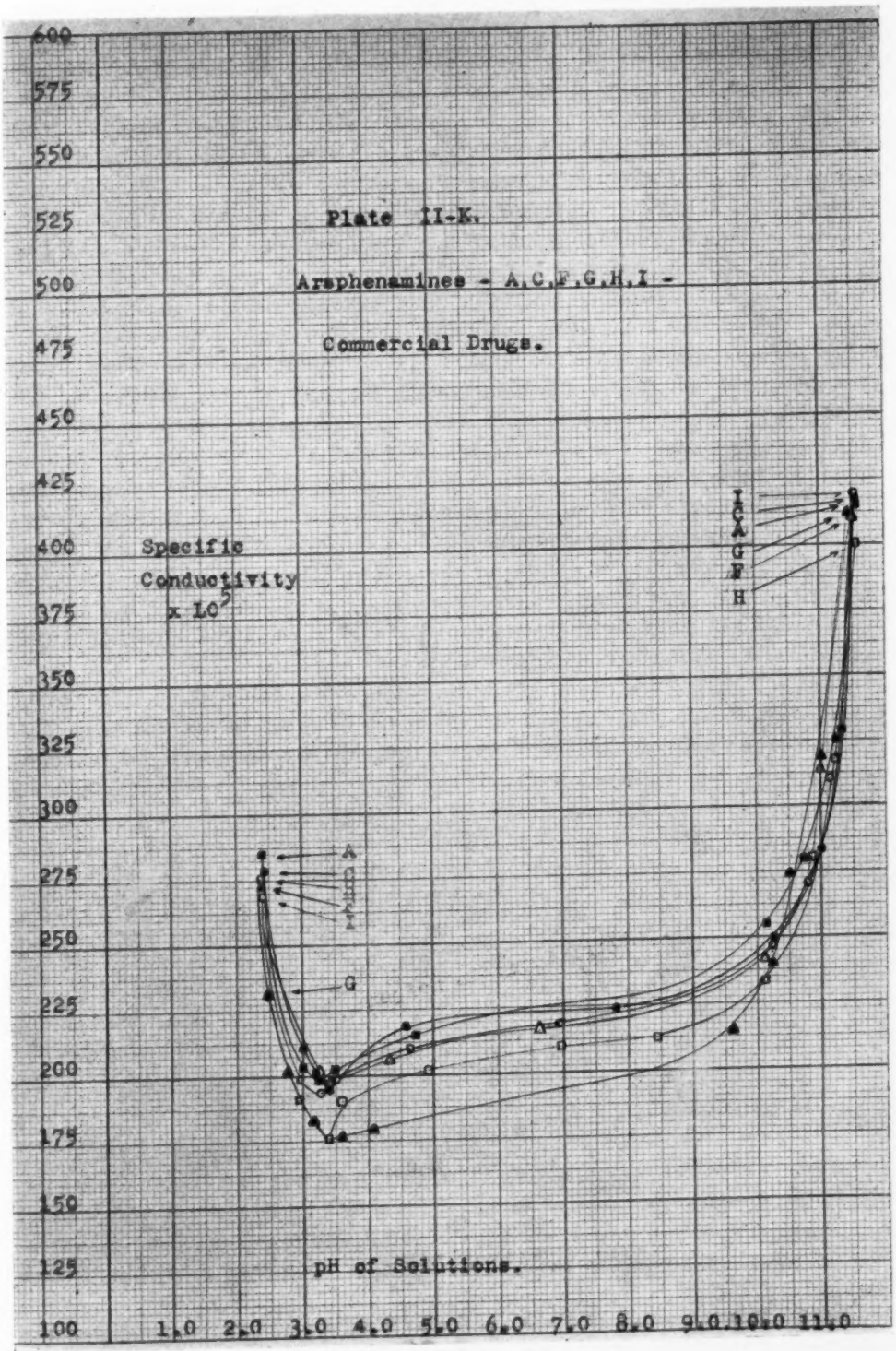


Plate II-K.
Arsphenamines - A,C,F,G,H,I -
Commercial Drugs.
Specific
Conductivity
x 10^5
pH of Solutions.
600
575
550
525
500
475
450
425
400
375
350
325
300
275
250
225
200
175
150
125
100
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0 9.0 10.0 11.0
I
C
A
G
F
H
A
G

The mean value for the isoelectric point calculated from the eight normal samples studied is 3.39. The fact that this point is found so far over on the acid side indicates that the acid ionization of arsphenamine is much stronger than the basic ionization. It must be noted that the P_H values of the dihydrochloride solutions as well as of the alkalinized solutions are remarkably in agreement for the different arsphenamines. This would seem to indicate that arsphenamine, when correctly prepared, contains the same amount of acid, regardless of the method of manufacture. Indeed, when a solution of the dihydrochloride was made up and to it added 1.45 c.c. 0.1 N HCl per 0.1 gm. sample (2 molecular equivalents from the point of minimum conductivity) the P_H of the solution dropped to 2.047. This would mean that the presence of excess acid was not covered by any buffer action of the drug. An interesting thing in these curves is the slope after the minimum has been passed. It is to be observed that this rapid change in the P_H value of the solution occurred through the region of precipitation which expends approximately from a P_H of 3.0 to about 10.5 (Plate II-C). The variability of this region is further shown by Table XVI, where it can be seen that the P_H values of the comparative solutions of the different arsphenamines are very uniform with the exception of the solutions which contained 4.0 c.c. 0.1 N NaOH per 0.1 gm. These solutions are about in the middle of the precipitation region. The materials appear to fall in two groups: Arsphenamines A, C, G, and H having a mean P_H value of 8.53 while the mean value of B, D, E, F and I is 6.63. It is at this point that the most marked difference appears.

Table XVIII gives the alkalinization data for arsphenamine in terms of the P_H values. As these values are the mean of those of six different commercial drugs and two laboratory preparations they may well be taken as representative of arpshenamine.

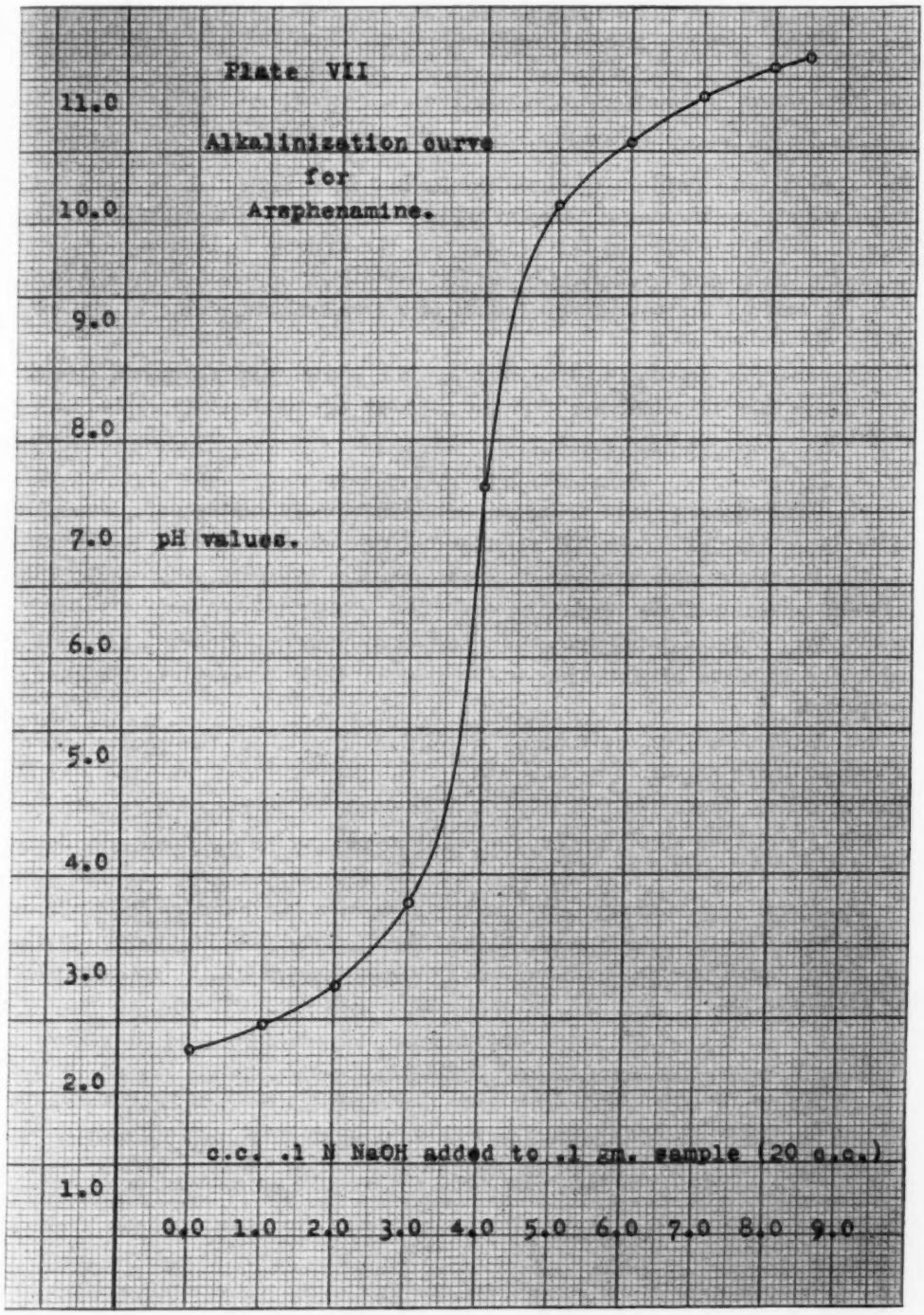
TABLE XVIII

ALKALINIZATION TABLE FOR ARSPHENAMINE

C.C. 0.1 N NaOH ADDED PER 0.1 GM. ARSPHENAMINE	P_H VALUE OF SOLUTION
0.0	2.410
1.0	2.630
2.0	2.980
3.0	3.740
4.0	7.580
5.0	10.170
6.0	10.740
7.0	11.170
8.5	11.530

Plate VII which was made up from this data may then be taken as the alkalinization curve for arsphenamine. This shows how the P_H of the solution changes during alkalinization upon preparing a solution of the drug for intravenous injection according to the specifications of the U. S. Public Health Service.

From this information it was possible to calculate the P_H values of the solutions of the various salts of arsphenamine. The arsenic content of the preparations examined ranged from 30.80 to 32.3 per cent. Taking a mean



value of 31.34 per cent As. (theory = 34.17 per cent), a 1.0 gm. sample made up to 200 c.c. corresponds to a 0.014 molal solution. On this basis the P_H values of the salts of arsphenamine are as follows:

Dihydrochloride	= 2.41
Monohydrochloride	= 3.00
Arsphenamine base	= 7.60
Monosodium salt	= 10.88
Disodium salt	= 11.43

The approximate position of these points has been indicated on Plate I-C. These values are in good agreement with those of Elvove and Clark (1924). In addition they confirmed the accuracy of the hydrogen electrode measurements by the rate of hydrolysis of methyl acetate in the case of the dihydrochloride and by the rate of hydrolysis of nitrosotriacetonamine in the case of the alkalinized solution.

A general discussion will be reserved for a later paper wherein this investigation has been carried over to a study of alkalinized arsphenamine solutions.

SUMMARY

1. The hydrogen electrode measurements have been made on arsphenamine solutions and are found to give reliable and reproducible data.

2. A radiotron vacuum tube has been used as a generator of alternating current for conductivity work.

3. Eight different brands of commercial arsphenamine have been studied with the purpose of detecting differences in their physical and colloidal properties.

4. Variations of P_H were found in solutions after about two equivalents of alkali had been added. The solutions of the dihydrochloride and the alkalinized solutions showed, in general, but little variation in their P_H values.

5. A practical alkalinization curve for arsphenamine has been established in terms of P_H values.

6. A uniform, minimum conductivity measurement indicates the presence of an isoelectric point of arsphenamine at the P_H about 3.4. Reproducible conductivity measurements were made on solutions throughout the entire process of alkalinization.

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FURTHER OBSERVATIONS ON A CHEMICAL FACTOR IN THE RESISTANCE TO TUBERCULOSIS*

By H. J. Corper, M.D., Ph.D., and Max B. Lurie, M.D., Denver, Colorado

IT is at present generally acknowledged that tubercle bacilli gaining entrance into the animal economy do not necessarily produce tuberculosis there, but the mechanism whereby the disease-producing propensity of this organism is prevented from manifesting itself is rather obscure. There are, however, factors, primarily of cellular nature and necessarily influenced by the virulence of the infecting bacilli which, though insusceptible of crucial experimental test, must be acknowledged as of importance as a determinative in turning infection into disease and may account for the difference in species susceptibility to tuberculosis.

The preponderance of pulmonary tuberculosis among the human races has led to many suggestions in explanation for its prevalence, *viz.:* a diminished resistance of the lung over other organs (a veil for our ignorance); the lung acting as a bacterial filter (Borrel, Aufrecht); the greater incidence of aerogenic infection (Cornet, Flügge); the absence or comparative weakness of pulmonary lipases; the peculiar relation of the pulmonary circulation (White); and yet none have been susceptible to crucial experimental tests nor has this been successful when tried. That the lung is especially susceptible to tuberculosis spontaneously in man and animal is an accepted universal fact, and experimentally one of the most striking evidences of pulmonary susceptibility is presented in the differentiation test for human and bovine tubercle bacilli by intravenous injection into rabbits as disclosed by the epoch-making discovery of Theobald Smith in 1898,[1] although the organ deposition following such injections was not seriously considered in these studies. Smith even states that "the lungs, as the first and chief recipients of the injected bacilli, were in a state of expansion (apparently due to tuberculosis), which in some cases was extreme," thus showing his complete failure to realize at that time the localization of the bacilli in the body. Following the intravenous injection of human tubercle bacilli the disease is limited almost exclusively to the lungs, while bovine bacilli although producing a general disease also prefer a pulmonary localization in these animals (Oehlecker, Askanazy, Donati).

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In 1921, Corper, Gauss and Rensch[2, 3] pointed out that humoral antibodies have failed to explain resistance to tuberculosis; the lymphocyte as the important factor is rapidly being discarded; allergic phenomena require further elucidation, and the phagocytic action of the wandering endothelial cell is recognized as important; but both humoral and cellular mechanisms of resistance leave much to be explained. They believed the empiric reasoning that the deficiency of carbon dioxide in the body favors tuberculosis, while an accumulation retards it, seems to be highly significant. In experiments conducted at that time they found that three per cent carbon dioxide inhibits the growth of tubercle bacilli in the test tube and fifteen per cent is tuberculocidal. Cultures of tubercle bacilli buried in the tissues of animals and permitted to acquire the carbon dioxide concentration of the body are definitely inhibited in their growth, while other cultures similarly buried, except that ingress of atmospheric air is permitted, show no inhibition. It appeared from these experiments that carbon dioxide may play a significant rôle in the resistance to tuberculosis.

In 1924, Corper and Goldberg[4] reported on experiments in rabbits in which they found that the carbon dioxide content of the blood plasma obtained from a congested auricle, produced by applying mild constriction of rabbit's ears, was about 10 to 20 per cent more in terms of bicarbonate than that from normal auricles; and that tubercles resulting from the intracutaneous inoculation of graded amounts of virulent human and bovine tubercle bacilli, tended to ulcerate and discharge their contents earlier in the congested than in the noncongested auricle of the same rabbit. The development of local tubercles was retarded by prolonged constriction of the base of the auricle, with its resultant continuous mild congestion and edema. This effect was not discernible when heat-killed bacilli were used. Prolonged mild constriction of the auricle had no appreciable influence on the development of multiple metastatic lesions resulting from infection with virulent bovine bacilli nor on the development of tuberculosis in the regional lymph nodes of the ear after infection with virulent human or bovine bacilli. About this same time Levinson and Petersen,[5] reporting on the effect of passive hyperemia of the liver on tubercle formation, found that in an experimentally produced passive hyperemia of the liver, no striking differences were noted grossly between the treated and control animals as to the quantitative distribution or size of tubercles in the liver; but histologic examination of the livers showed a greater deposit of fibrous tissue around and within the tubercles in animals that received oil before and after being injected with tubercle bacilli. The lungs of the control animals contained more tubercles than the lungs of the animals receiving oil after the tubercle bacilli injection. The lungs of the rabbits receiving oil, prior to tubercle bacilli injection, were relatively free from tubercles. Admittedly these experiments are complicated by the use of the oil and the authors comment on the findings noted but offer no explanation for them.

In considering the action of particulate substances such as oil and carbon given intravenously, it seems pertinent that the distribution of these materials in the animal economy be considered. This has been fairly well studied

with various types of rapidly growing bacteria, carbon and relatively insoluble chemical substances but it seems to have been overlooked or ignored in considering bacterial infections. However, in 1908 Oettinger,[6] in studying the disposition of the lung to tuberculosis in furtherance of Flügge's views, pointed out that after entrance of tubercle bacilli into the blood stream primarily the lungs alone become diseased. This he attributed not to a greater disposition of the lung to retain the bacilli but to an increased disposition of the lung tissues to react with disease to a smaller number of bacilli. Bacteria, circulating in the blood like other corpuscular elements, are deposited in all the organs of the body but in far less amount in the lungs than in the spleen and liver. If bacilli after ingestion are found in the lungs while the liver and spleen remain free from them it is certain that they did not enter the lungs via the blood stream. Oettinger points out the erroneous conceptions held by Borrel in 1893 and Aufrecht in 1905 that the lungs acted as a selective filter for tubercle bacilli introduced into the blood which supposedly accounted for the development of the tuberculosis there.

Oettinger used rabbits to which were given intravenous injections of bacillary suspensions of B. prodigiosus, Staphylococcus albus, hog erysipelas, mouse typhoid and typhoid bacilli. Unfortunately his studies were only over a maximum of four hour intervals. The relation of the lung, liver and spleen in bacterial content was found to be extremely variable ranging from 1:1200:260, with prodigiosus, to 1:15:12 with mouse typhoid at one hour, and from 1:3:80 with prodigiosus, to 1:100:166 with typhoid at four hours interval. An attempt to study the organ distribution of tubercle bacilli after intravenous injection into the ear vein of the rabbit and using the dilution extinction method as an index to the number of bacilli with the guinea pig as test animal, yielded unsatisfactory results, since in none of the dilutions used was infection absent in the guinea pigs and the conclusions drawn were that the lung, liver and spleen were all heavily seeded with tubercle bacilli after intravenous administration. With timothy hay bacilli, however, and using plating methods the spleen and liver were found to contain twenty times as many bacilli per gram as the lung. To overcome the criticism that small numbers of bacilli may lodge mainly in the lungs, this was tried using 1000 and 10,000 B. prodigiosus but the relations still bore out the fact that the liver and spleen gave colonies while the lung usually did not.

Regarding the quantitative distribution of particulate material after intravenous administration, the recent excellent work of the Harvard investigators, Drinker and Shaw,[7] Lund, Shaw and Drinker[8] and Drinker, Shaw and Drinker,[9] furnish about the best available information and especially so, because the study included a number of different animals, *viz.*, the dog, rabbit, guinea pig, cat, rat, chicken and turtle. Manganese dioxide and manganese metasilicate were chosen because of their ease of exact chemical determination and the natural absence from the tissues. In brief, these observers found that particles no larger than one micron disappeared completely from the blood of the cat in one hour and at that time the lungs contained 47 per cent, liver 38.3 per cent and spleen 4.3 per cent. The cat is, however, an exception, and in dogs, rabbits, guinea pigs, rats, chickens and turtles the liver receives

the greater part of the particulate matter. In the cat, after a period of twelve hours, the liver accumulates the manganese which was formerly deposited in the lungs. The relative solubility of manganese dioxide may play a part here. In rabbits, after one hour, the lungs contained 1.6 per cent of the injected manganese metasilicate and the liver 88.2 per cent, while after sixteen days the relation still held in that the lung contained 0.6 per cent and the liver 32.4 per cent.

There are a large number of observations on the organ distribution of carbon following intravenous injection but most of them are inaccurate and gauge the amount of carbon by placing the organs side by side and grading them on optical appearances. Thus in a recent contribution, Wislocki[10] found that carbon particles from one to three microns in size are usually more prevalent in the lungs of rabbits, to less extent in the liver and spleen especially, diminishing gradually up to twenty days. It is believed that the deposition of carbon in the lungs is not the result of simple filtration by the capillaries. In the cat, carbon particles were retained in greater number in the pulmonary circulation than those in the rabbit. Likewise Corper, Starry and Lurie,[11] in studying the effect of carbon on tuberculosis in the rabbit, record that carbon administered in liquid suspension intravenously daily for long periods, deposits mainly in the liver and spleen and to less extent in the lungs but in sufficient amount to have a retarding influence upon the development of miliary pulmonary tubercles in these animals.

The most complete study on the fate of India ink injected into the blood is reported by Nagao of McCormick Institute for Infectious Diseases in 1920 and 1921,[12, 13, 14] whose purpose it was, by this means, to throw light on the distribution and fate of microbes that invade the blood. Unfortunately his observations made on rabbits and guinea pigs were not quantitative and judgment was based on gross and microscopic appearances only, yet he corroborates the earlier findings in that the liver, spleen and bone marrow were intensely black, while the small intestines and the lungs were stained blackish only after large doses. The liver retained much of the carbon for more than a year, although its localization changed slightly during the interval; the carbon being found in the endothelial cells of this organ. In the spleen the endothelial cells of the capillaries and sinuses, as well as the splenocytes, took up ink granules while the malphigian bodies were practically free from ink in the early stages. The spleen seemed to acquire more granules as time went on, while in the marrow, as well as in the liver, the amount of ink granules seemed to decrease. In the lung, after moderate doses of ink suspension, granules were found in the endothelial and mononuclear cells, but no ink emboli were found except after large injections. In this organ the amount of granules found was always rather small and only a few masses. Eventually the granules of ink disappeared almost wholly from the lung.

Apparently ink granules are not discharged from the body by any particular organ after injection into the blood, but it is possible that they are carried to the outside by emigrating phagocytes. It is stated that the fate of tubercle bacilli, when introduced into the blood, was being studied, but a careful survey of the literature since that time has revealed no article by this

author on this subject. It is possible that the technical difficulties encountered in such a study has led to abandoning it.

A great many studies of the fate of intravenously injected bacteria have been only crudely exact and bear out for the shorter interval studies the distribution noted for carbon. For longer intervals other conflicting factors enter, such as bacteriolysis, agglutination, rapid growth, etc., which naturally are an insignificant consideration when dealing with a slow-growing microorganism like the tubercle bacillus. In dogs, Bartlett and Ozaki[15] found that the Micrococcus aureus injected into the left ventricle of the heart was almost immediately stored up in relatively large numbers in the lung capillaries, being at first chiefly extracellular; there it is rapidly ingested by polymorphonuclear leucocytes. Shortly after injection, the cocci are stored up in the liver and spleen, their presence there, in considerable numbers, being coincident with their decrease in the lung. In the liver and spleen they are found largely in wandering cells and in fixed cells, in both of which they rather quickly undergo bacteriolysis. The bacteria completely disappear from these organs within forty-eight to seventy-two hours. Ito[16] found bacteria, Staphylococcus pyogenes aureus, that accumulate in the lungs in the earlier stages, are transported to other organs. Hopkins and Parker[17] give more accurate figures for the distribution of hemolytic streptococci; ten minutes after injection into rabbits, in 0.1 gram tissue the lung contained 21,000 to 41,000, the liver 104,000 and the spleen 120,000 cocci by plating methods.

In pursuance of the Bartel conception that certain tissues of the body possess the power to reduce the virulence of tubercle bacilli,[18] Neumann and Wittgenstein[19] gave eight female dogs intravenous injections of suspensions of tubercle bacilli and examined the blood and organs (lungs, bronchial lymph glands, mesenteric lymph glands, liver, spleen and ovaries) after intervals of half an hour, twenty-four hours, three, seven, eleven, twenty-two and thirty-five days by guinea-pig inoculation, histologically and by culture methods. Tubercle bacilli were found in the blood and all of the organs except the ovaries up to thirty-five days. Histologically, the lungs reacted with the formation of tubercle at seven days while the liver, spleen and ovaries showed no evidence of tubercle at this time but did after eleven days. In a number of dogs the lungs showed distinctly more involvement. The lung alone of all the organs was found to have no influence upon the virulence of the bacilli. In this study the authors apparently confused virulence with an actual increase in the numbers of bacilli. They finally attribute the disposition of the lungs to the tuberculosis to the fact that lung tissue *in vitro* has little effect upon the bacilli as compared with other organs and believe that this is accounted for by the development of epithelioid cell tubercles and the absence of a fat splitting enzyme in the lung. The fact that the number of bacilli may have had something to do with the size of the tubercles did not enter their minds.

A large number of observers have noted the tendency to the development of pulmonary tuberculosis following the intravenous injection of tubercle bacilli in animals, and some have suggested studying the correlation between the primary lodgment and the disease, but probably on account of the technical

difficulties encountered no one seems to have reported on this phase in tuberculosis, especially from an approximate quantitative standpoint and by using several different animal species. This was the primary purpose of the study to be briefly reported here. The animals chosen were the rabbit, guinea pig and dog. The cat was not used because all available evidence indicated that the lungs in this animal were seeded more heavily with particulate matter given intravenously than the liver and spleen. On account of the difficulty encountered in determining with any degree of accuracy the numbers of tubercle bacilli in an organ and the apparently similar organ deposition of many forms of particulate matter in the same animal, carbon was chosen as a gauge for the deposition, primary and subsequent, of the tubercle bacilli injected intravenously.

THE IMMEDIATE AND FINAL ORGAN DEPOSITION OF CARBON FOLLOWING INTRAVENOUS INJECTION

For the purpose of determining the immediate and final organ distribution of carbon, guinea pigs, rabbits and dogs were given intravenous injections of India ink, in which the particles were approximately about one micron in size and by volume would give a sediment, after centrifugation at 3000 r.p.m., of about 0.2 c.c. carbon in 1.0 c.c. of original ink which was equivalent to a sediment of tubercle bacilli obtained from a suspension of 100 mg. Besides noting the gross and microscopic distribution of the carbon in the three main organs, lung, liver and spleen, which will be included in this report, the carbon content of the organs was determined colorimetrically after completely digesting the tissues with hot 40 per cent potassium hydroxide and sedimenting the carbon after two washings with an excess of 95 per cent alcohol, and further washing the sediment with two changes of ether and finally suspending the residue in 0.9 per cent sodium chloride solution, which suspension was compared in a colorimeter with a known suspension of India ink in saline. This method was accurate to 0.001 c.c. of India ink (0.000,2 c.c. of solid carbon suspension) per gram of tissue. Beyond this point, colored substances derived from the tissues themselves interfered with the readings.

By means of this method, in a series of thirty-four rabbits given intravenous injections of 3 c.c. of 1:3 dilution of India ink per pound of body weight, and analyzing the spleen, liver and lung for carbon content after intervals of thirty minutes, three hours, one and four days, one and two weeks and one month it was found that in all the above-mentioned time intervals after intravenous injection of the India ink, except the thirty minute interval, the lungs invariably contained less carbon per gram of tissue than the liver and spleen of the same animal. In the thirty minute interval, the lungs of the rabbits contained more carbon than the liver though less than the spleen per gram tissue. These relations are shown in Table I, which are the average figures obtained from the entire series of animals.

With the same dose of India ink per pound animal weight given intravenously, there was found a wide variation between individual rabbits but

TABLE I

THE RELATIVE AVERAGE DISTRIBUTION OF CARBON IN THE LUNG, LIVER, AND SPLEEN OF RABBITS AT DIFFERENT INTERVALS AFTER THE INTRAVENOUS INJECTION OF INDIA INK*

ORGAN	AMOUNT OF CARBON (IN CUBIC CENTIMETERS OF EQUIVALENT OF ORIGINAL INDIA INK) CONTAINED IN 1 GRAM OF ORGAN ANALYZED AFTER:—						
	30 minutes	3 hours	1 day	4 days	1 week	2 weeks	1 month
Lung	0.04	0.02	0.02	0.009	0.02	0.009	0.004
Liver	0.01	0.03	0.03	0.03	0.03	0.03	0.009
Spleen	0.07	0.07	0.14	0.07	0.21	0.11	0.02

*There was noted no appreciable difference in the distribution of the carbon in these organs whether a fine suspension of carbon (particles of about 1 micron size) or slightly coarser (particles of about 3 micron size) particles were used for intravenous injection.

the relation between the carbon found in the lung, liver, and spleen was found to be almost invariable in the same animal.

Similar analyses of the organs of guinea pigs given India ink (about 4 c.c. of 1:6 dilution per pound) and analyzed after thirty minutes, three hours, one and two days, two weeks, and eight months, revealed that at the intervals examined the lungs always contained less carbon per gram weight than the liver or spleen. The ratio between the lung, liver and spleen was about 1:10:20, although this varied for the individual animal. In no case examined,

TABLE II

THE RELATIVE AVERAGE DISTRIBUTION OF CARBON IN THE LUNG, LIVER, AND SPLEEN OF GUINEA PIGS AT DIFFERENT INTERVALS AFTER THE INTRAVENOUS INJECTION OF INDIA INK

ORGAN	AMOUNT OF CARBON (IN CUBIC CENTIMETERS OF EQUIVALENT OF ORIGINAL INDIA INK) CONTAINED IN 1 GRAM OF ORGAN ANALYZED AFTER:—					
	30 minutes	3 hours	1 day	2 days	2 weeks	8 months
Lung	0.001	trace	trace	0.006	0.007	0.002
Liver	0.02	0.01	0.01	0.01	0.01	0.005
Spleen	0.01	0.03	0.03	0.03	0.02	0.01

however, did the liver or spleen contain an equal or less amount of carbon per gram weight than the lungs. Comparatively speaking the lungs of the guinea pig contained less carbon per gram of tissue with relation to the liver and spleen than that noted for the rabbit. The averages of analyses of the organs of seventeen guinea pigs are recorded in Table II.

TABLE III

THE RELATIVE AVERAGE DISTRIBUTION OF CARBON IN THE LUNG, LIVER AND SPLEEN OF DOGS AT DIFFERENT INTERVALS AFTER THE INTRAVENOUS INJECTION OF INDIA INK

ORGAN	AMOUNT OF CARBON (IN CUBIC CENTIMETERS OF EQUIVALENT OF ORIGINAL INDIA INK) CONTAINED IN 1 GRAM OF ORGAN ANALYZED AFTER:—					
	30 minutes	3 hours	1 day	4 days	1 week	1 month
Lung	0.03	0.01	0.03	0.03	0.05	0.01
Liver	0.05	0.02	0.03	0.04	0.05	0.04
Spleen	0.03	0.04	0.05	0.03	0.05	0.08

Analyses of the organs, lung, liver and spleen of twelve dogs given India ink (2 c.c. of 1:2 dilution in saline per pound of body weight) intravenously after thirty minutes, three hours, one and four days, one week, and one month, revealed that the lungs per gram weight tend to retain less of the carbon

than the liver and spleen, although this did not occur as consistently as in the guinea pig or rabbit. There was also lacking the striking differences between these three organs with relation to the amount of carbon retained following the intravenous injection of India ink. The liver and spleen at times assumed a reverse relationship in that the liver would retain more carbon than the spleen. However, in all the animals, rabbits, guinea pigs and dogs, there was a gradual removal of the carbon from all three organs as time went on, although this occurred very slowly. The average of the analyses at the different intervals for the dogs is recorded in Table III.

Considering the fact that the guinea pigs received intravenously about two-thirds c.c. of full strength India ink per pound, the dog and rabbit 1 c.c. per pound, it is possible to compare in a general way the relative pulmonary

Fig. 1.—Distribution of tuberculosis in the guinea pig four weeks after the intravenous injection of 0.1 mg. virulent bovine tubercle bacilli. Note the absence of liver and pronounced pulmonary involvement.

localization in these animals; the dog's lung received most, the rabbit's lung an intermediate amount and the guinea pig's lung the least on the basis of 1 gram tissue weight. However, in all these figures the amount of carbon in the actual lung volume is exaggerated since 1 gram lung tissue considerably exceeds the volume of 1 gram liver or spleen tissue.

THE TUBERCULOUS INVOLVEMENT OF VARIOUS ORGANS FOLLOWING THE INTRAVENOUS INJECTION OF TUBERCLE BACILLI

For the purpose of studying the tuberculous involvement in rabbits following the intravenous injection of suspensions of tubercle bacilli, thirty rabbits were given 1.0 mg., 0.01 mg., 0.000,01 mg. and 0.000,000,1 mg. of a

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uniform suspension of virulent bovine tubercle bacilli. The bovine tubercle bacillus was used in rabbits because of the comparative greater susceptibility of these animals to the bovine bacillus over the human bacillus, and the acknowledged general selective pulmonary election of the human bacillus following intravenous injection in these animals. The animals were examined at various intervals from two weeks to three months after intravenous injection.* In the 1.0 and 0.01 mg. dose of bacilli twelve of fourteen rabbits revealed

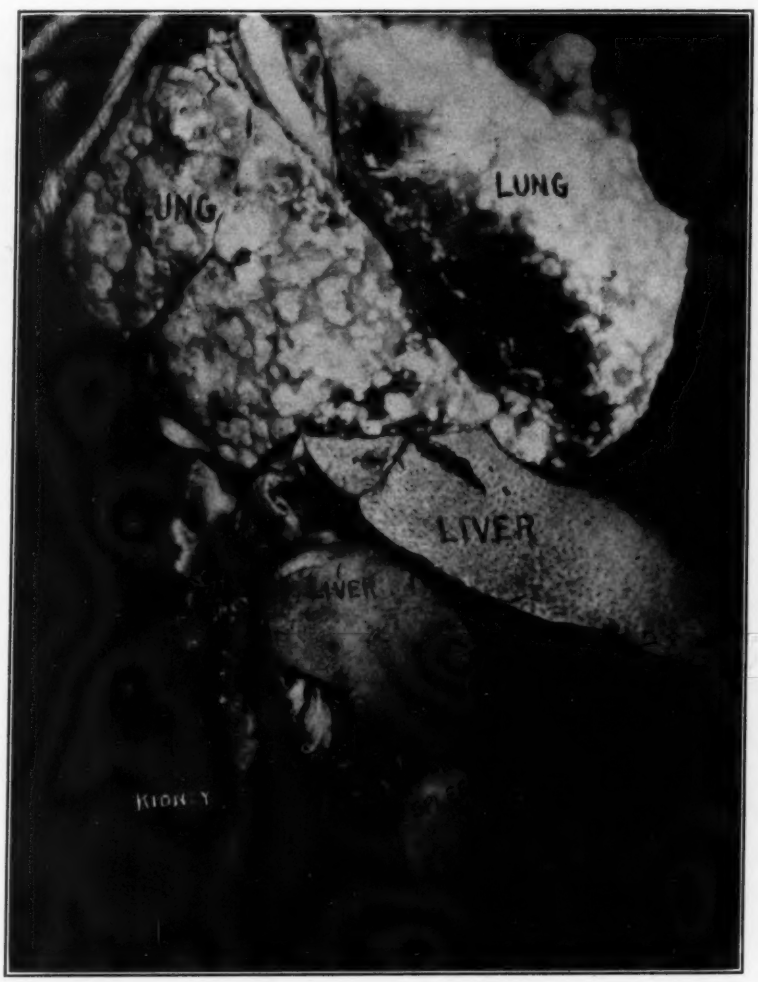

Fig. 2.—Distribution of tuberculosis in the rabbit eleven weeks after the intravenous injection of 0.000,01 mg. of virulent bovine tubercle bacilli per pound body weight. Note marked pulmonary involvement, slight splenic involvement and absence of hepatic involvement.

a massive involvement of the lungs and of these only one showed a slight macroscopic tuberculosis of the liver, and the spleen was involved slightly or moderately in seven of the twelve rabbits, while the kidneys were slightly to moderately involved in eight. In the remaining two rabbits there was a

*For lack of space the elaborate tabulated data on the tuberculous involvement of the rabbits, dogs and guinea pigs following the intravenous injection of tubercle bacilli will be recorded more fully in a subsequent article elsewhere.

miliary pulmonary tuberculosis with no macroscopic involvement in any of the other organs examined. Of the sixteen rabbits that received 0.000,01 and 0.000,000,1 mg. of bacilli, the lung was massively involved in three of the animals, while the liver revealed a slight involvement in only one of these, the spleen of two was moderately involved and one had a slight involvement. The lungs of four rabbits contained numerous large individual tubercles and in only one of these was the liver moderately involved while the spleen was only slightly involved in all four. The lungs of two rabbits were moderately involved with no hepatic or splenic involvement macroscopically. The lungs

Fig. 3.—Distribution of tuberculosis in the dog thirteen weeks after the intravenous injection of 0.001 mg. of virulent bovine tubercle bacilli per pound body weight. Note presence of pulmonary miliary tubercles and absence of involvement of the spleen and liver.

of seven rabbits were slightly involved with splenic involvement in one case only.

The experiments in rabbits indicate the remarkable susceptibility of the lung to tuberculosis as contrasted with the remarkable resistance of the liver in this animal, the spleen being intermediate in susceptibility. It was also noted that the kidney was involved in the same number of animals as the spleen. The size of the tubercles in these two organs tended to be the same—the difference between the number of tubercles in the spleen and kidneys being accounted for by the relative seeding. The spleen in the rabbit does not enlarge greatly when involved with tuberculosis, while in the guinea pig it does.

In order to study the tuberculous organ involvement in guinea pigs following the intravenous injection of suspensions of tubercle bacilli forty-four guinea pigs were given 0.1, 0.001, 0.000,01 and 0.000,000,01 mg. of a suspension of virulent bovine tubercle bacilli and groups of these animals were killed two, four and six weeks after injection. In addition, twelve guinea pigs were given intravenous injections of a suspension of virulent human tubercle bacilli in doses of 0.1, 0.001, 0.000,01 and 0.000,000,01 mg. and were examined two, four and six weeks after injection. From a critical study of the data

Fig. 4.—Distribution of tuberculosis in the dog seven weeks after the intravenous injection of 0.001 mg. virulent human tubercle bacilli per pound body weight. Note the larger size of the miliary tubercles in the lung, in distinction to the greater number of those in the liver; the number apparently indicating the relative seeding of the bacilli in these two organs when both organs develop the disease.

obtained from these animals having received human or bovine bacilli, it was noted that during the first two weeks macroscopic tubercles appeared in the lungs of seventeen of twenty guinea pigs without macroscopic evidence of their presence in the liver; in the remaining three, slight involvement occurred but the tubercles were much smaller than those seen in the lungs of the same animals. After four weeks the guinea pigs fall into three classes,

those having received 0.1 and 0.001 mg. of bacilli uniformly had more macroscopic tuberculosis in the lungs than in the liver while those having received 0.000,01 mg. had more macroscopic tuberculosis in the lungs in three of five animals, while one had the same amount and one appeared to have more in the liver, and those having received 0.000,000,01 mg. had approximately the same number of tubercles in both lung and liver but in all the guinea pigs examined after four weeks the individual pulmonary tubercles were larger in seventeen of twenty animals than the corresponding tubercles in the liver; in two the size was the same and in one the tubercles in the liver were larger, which exceptions occurred only in the smaller doses. The tendency seems to be borne out that the more the pulmonary involvement the less the liver involvement. All the guinea pigs receiving 0.1 mg. of bovine bacilli died before the six weeks interval but those having received the human bacillus lived for the entire interval of time. Of the bovine virulent set at six weeks, three of four receiving 0.001 mg. had more hepatic than pulmonary involvement, three of four receiving 0.000,01 mg. and one of four receiving 0.000,000,01 mg. With the human bacillus the lung was selectively involved in all doses except in the smallest dose (0.000,000,01 mg.) in which case the number of tubercles were approximately equal but the pulmonary tubercles were distinctly larger. After six weeks, there was a tendency for the development of more tuberculosis in the liver than in the lung when virulent bovine bacilli were injected. With the human bacillus there was still more tuberculosis in the lungs even after six weeks.

The experiments on dogs included a series of thirty dogs, twenty-two given intravenous injections of 0.5 mg., 0.1 mg., 0.001 mg., 0.000,01 mg. of virulent bovine tubercle bacilli per pound weight and eight dogs were given the same doses of virulent human tubercle bacilli. The animals were examined at intervals of twenty-two days to 146 days after the intravenous injection of suspensions of the bacilli.

In every case of the thirty dogs the individual macroscopic tubercles in the lungs were larger than the macroscopic tubercles in the liver of the same dog. In twenty of the thirty dogs the total number of tubercles for a given area of lung were more numerous than a corresponding area in the liver of the same dog. In six of the thirty dogs, the liver contained more numerous tubercles than the lung of the same dog. The six dogs in which the tubercles were more numerous in the liver than in the lungs would seem to verify the distribution law established with carbon for tubercle bacilli. In fifteen of the thirty dogs, there was no macroscopic tuberculosis in the liver while there was definite macroscopic tuberculosis in the lungs.

DISCUSSION AND CONCLUSIONS

All the evidence available to date indicates that the distribution of particulate material of sufficiently small size not capable of producing capillary embolism following the intravenous injection into guinea pigs, rabbits and dogs, favors deposition in the liver and spleen of these animals as compared to the lung, both as to optical appearance and according to chemical analyses on the basis of unit weight (or volume) of organ examined. On purely *a priori*

reasoning, and in the absence of contradictory evidence, it seems that tubercle bacilli, in fine suspension injected intravenously, deposit in these organs in relatively the same proportions. If tuberculosis develops at all in the organs of these animals, it develops only in those organs which contain macroscopic carbon and an appreciable amount by chemical analyses. When the liver of these animals does not exercise a complete inhibitory action on the development of the tubercle bacilli in this organ, the relation between the number of tubercles in the lungs and liver closely correlate the deposition findings with carbon. Krause[20] has concluded from anatomic studies that the lung of the guinea pig retains less tubercle bacilli than that of the rabbit, following entrance of the bacilli into the circulation. This fact is directly borne out by the deposition of the carbon in our studies and the subsequent development of the tuberculosis in these two animals in that the relation between the liver and lung deposition in the rabbits is about 3 to 2, while in the guinea pig it is about 10 to 1, and similarly for the tuberculosis, although this is complicated by other factors.

It is also noteworthy that the microscopic study of all the organs reveals that the localization of the early tubercles in the lungs, liver, spleen and kidney is the same as the deposition of the carbon following intravenous injection. This is also borne out by the reports of the investigators who studied the immediate distribution of the rapidly growing bacteria following intravenous injection.

Therefore, it seems justified to conclude that the distribution of carbon following intravenous injection is a good approximate index of the distribution of the tubercle bacilli following intravenous injection.

If this is admitted there is a fundamental discrepancy between the organ development of tuberculosis and the bacillary distribution following intravenous injection. In the rabbit, while the bacilli are deposited in definitely smaller numbers in the lungs than in the liver and spleen, the tuberculosis, when it develops following the intravenous injection of human or bovine tubercle bacilli, develops in the lungs to a far greater extent than in the liver and spleen; in fact it is rare to have macroscopic tuberculosis develop in the liver of these animals, while the spleen occupies an intermediate position between these two organs in amount of tuberculous involvement. In the guinea pig, especially during the early periods of infection, two and four weeks, the evidence again is in favor of a greater susceptibility of the lungs of these animals over the liver. This general rule of greater pulmonary over hepatic susceptibility to tuberculosis also is borne out by the studies on the dogs. These observations are in entire accord with the conception of Oettinger of a special disposition of the lungs to tuberculosis.

Attempts to explain pulmonary disposition to tuberculosis are numerous, including the obviously wrong conception of increased deposition of bacilli in this organ following entrance into the blood stream, a virulence destroying property of this organ,[21] and the lack of pulmonary lipases. It is conceivable that cellular factors might explain the relative organ differences in these animals but the striking pulmonary susceptibility in distinction to the hepatic resistance in all of them and the intermediate uniform tuberculosis of the

kidney and spleen—especially in the rabbit and dog—dependent upon the primary deposition of the bacilli following intravenous injection, would seem to indicate that another factor is active in determining the results in all the animals studied, which is the same for all of them. That the greater motility of the lung over all the other organs does not enter in this is well established by the fact that unilateral artificial pneumothorax in the rabbit had no effect upon pulmonary miliary tuberculosis.[22] While all the organs considered vary as to their cellular structure, the lung and liver have one common variable and this is the blood supply. The lung is primarily an oxygenating and decarbonating organ for the body and as such distinguishes itself from all the other organs, while the liver, having essentially a venous circulation has the richest carbon dioxide and poorest oxygen supply of any organ in the body. In the light of the previous work on carbon dioxide and on the effect of constriction of the ear of the rabbit on tuberculosis developing in this appendage, it seems highly probable that this factor may be instrumental in determining pulmonary susceptibility to tuberculosis as opposed to hepatic resistance to the disease. In how far the oxygen content of these organs plays a rôle is not determined at present but is being studied. The fact, however, must not be lost sight of, that there is another important factor in tuberculosis accounting for a difference in species susceptibility which is also strikingly brought out by this study but which at present cannot be explained on a chemical basis.

From this point of view some of the most important facts in tuberculosis are easily understood—for instance, in the first place, the preponderance of pulmonary tuberculosis and relative rarity of other organ involvements in man and animals; and secondly, the age incidence and general conditions under which tuberculosis develops. Tuberculosis is a disease of early adult life, the time of greatest stress and strain associated with malnutrition, exhaustion and fatigue, all of which lower the alkali reserve of the body and thus its carbon dioxide retaining power. In the third place, rest and good nutrition, factors universally accepted as favoring the arrest of the disease, tend to increase the alkali reserve and hence the carbon dioxide combining power of the body. Fourth, the reduced incidence of tuberculosis among the poorer classes of society during the past hundred years can be correlated with the general improved living conditions of the people, shorter working hours and better nutrition; and fifth, the particular susceptibility to tuberculosis of individuals suffering from debilitating diseases—the diabetic is a classical example with lowered alkali reserve—and opposed to this the resistance to tuberculosis of the pregnant woman and those afflicted with certain cardiac and asthmatic conditions.

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ON THE RELATIVE SIGNIFICANCE OF A PARTIAL COMPLEMENT-FIXATION TEST WITH THE CHOLESTERINIZED ANTIGEN*

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THE practical application of the complement-fixation test by Wassermann, Neisser and Bruck[1] to the diagnosis of syphilis has successfully passed through the era of enthusiasm and the era of pessimism that followed, to establish itself as a specific reaction. And while it is accepted that a Wassermann test that exhibits complete inhibition of hemolysis is diagnostic of syphilis, we cannot bring ourselves to believe that a partial inhibition of hemolysis, especially +1, does not require or deserve a second thought.

It is not our desire to maintain that a partial complement-fixation test, particularly one where an alcoholic extract of a tissue antigen has been reinforced with cholesterin, would authorize the physician to entertain the deduction of the existence of syphilis in an individual whose physical examination and history do not begin to suggest that the patient is afflicted with syphilis. Nevertheless, inasmuch as syphilis is known at times to manifest itself in the disguise of some malady, the etiology of which is obscure; that histories as related by patients, although without intent to deceive, are often misleading, then a weakly positive serologic reaction which in the course of the study of the patient's case, is confirmed by relevant facts which bear witness that the patient's ailment is syphilis, assumes significance. That the test although only 1+ in intensity and performed with cholesterin antigen may possess diagnostic value, that it is not pure presumption but is susceptible to concrete demonstration, is exceedingly well exemplified by the case histories here presented.

Case 1.—M. J., white female, aged thirty-one years, housewife by occupation, was registered at the Gynecological Outpatient Department, May 6, 1922, she complained of hematuria.

Personal History.—Usual diseases of childhood. She began to menstruate at age of fourteen, married when seventeen years old and has had three apparently healthy children, aged thirteen, eight and three years respectively. Labor was normal. She denied luetic infection.

Present Illness.—In the summer of 1917, the patient noticed that there was some blood in the urine. She consulted a physician who irrigated the bladder and the bleeding "stopped." Thereafter the patient noticed the appearance of blood in the urine from time to time until several weeks prior to applying for treatment when the hematuria became constant.

Physical Examination.—The patient was fairly well nourished. The various examinations including the x-ray, the examinations upon the spinal fluid and the blood chemistry were negative. The cystoscopic examinations were made by Dr. H. Ginsberg who reported as follows:

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June 7, 1922, Mild infection of the trigone. Superficial ulcerations near the fundus. June 13, 1922, blood in the bladder. Both ureters catheterized. Bloody urine from left kidney. Urine from the right side clear. A routine Wassermann test was reported +1 with the cholesterinized antigen. The patient was referred to the dermatologic clinic where she was given a provocative test. Following this test the Wassermann reaction was reported +3.

The husband was then interviewed, but denied having been infected with syphilis. He submitted to a physical examination and a Wassermann test on the blood. The medical examination was negative but the Wassermann test was +4. When confronted with the positive test he admitted having had a chancre four months prior to his marriage. The oldest child was also examined and was found also to have a +4 Wassermann reaction.

Course.—Following the provocative test the hematuria cleared up. The patient has received four full courses of treatment—each course consisting of 4.8 gm. of neoarsphenamine and 12 gr. of mercury salicylate. At present the Wassermann test is negative. The patient since has given birth to a child which is physically and serologically negative.

CASE 2.—E. C., colored, female, aged eighteen years, referred to skin clinic from the Gynecological Outpatient Department where she was receiving treatment for gonorrhea. A routine Wassermann reaction was +1 with the cholesterinized antigen.

Following the provocative test, the serologic reaction was reported +4. The medical examination from the viewpoint of syphilis was negative. Consent for spinal drainage was refused.

Course.—The patient was irregular in attendance. The last test on the blood which was made in 1923 was still 4+.

CASE 3.—N. B., Italian, laborer by occupation, registered at skin clinic in 1921 with the express purpose to have his blood tested for syphilis, because, as he stated "one of the roomers at the boarding house was being treated for syphilis." The serologic examination on the blood was reported 1+ with the cholesterinized antigen. The routine medical examination was negative. The examinations on the spinal fluid were reported as follows: Wassermann reaction +4, cell count 31 per c.c., definite increase of globulin. Copper sulphate solution was reduced. The neurologic examination of the patient was made after findings on spinal fluid were known. The report of the neurologist was to the effect that the patient was free from mental and physical stigmata of syphilis.

Course.—Following the first treatment the intensity of the Wassermann reaction was reported 3+. After two years' treatment the blood Wassermann is negative. The Wassermann reaction on the spinal fluid is +1, cell count 7 per c.c., the globulin is not increased.

We believe that it would not be inappropriate to review briefly the events that led to the evolution of the use of cholesterinized antigen without discussing the subject of the Wassermann test at length. At present the serologic reaction is regarded as a synonym for the complement-fixation test for syphilis and is based upon the total or partial arrest of the otherwise inevitable destruction of the erythrocytes. When first formulated by Wassermann and his coworkers they believed that the test was a specific biologic reaction, between antibody and antigen. However when Marie and Levaditi,[2] Landsteiner[3] and Weil[4] were successful in obtaining an antigen from a large variety of normal tissues and even tumors, they demolished the belief that the reaction, from the standpoint of biology, is specific in nature. Wassermann then professed to believe that the reaction constituted a serum diagnosis of an antibody-like substance, the activity of which determines the fixation of the complement. The published researches of Landsteiner, Muller and Potz,[5] Porges and Meier,[6] in which they found that they could prepare an antigen by alcoholic extract from syphilitic and normal tissues, and that

their antigens possessed antigenic properties equal to those antigens that were prepared by extraction of syphilitic liver with physiologic salt solution, gave impetus to the study of substances other than tissue extracts that would have similar or superior antigenic properties.

A multitude of substances were experimented with. Fleishman[7] suggested the use of cholesterin. However it remained for Sacks[8] to make successful application of this particular lipoid substance. He was the first to add cholesterin to alcoholic tissue extracts and the results achieved gave Sacks the impression that in many respects the new antigen was the long sought and desired factor which made possible the achievement of a standard test. The superiority of cholesterinized antigen over the previously used antigens was soon confirmed by many independent investigators.[9]

The advent of protocols, in the medical literature, enumerate almost a legion of diseases other than syphilis that gave a positive serologic reaction; the admonitions that the cholesterinized antigen is too "sensitive" and is responsible for many false positives,[10] and the discrepancies in the reports of different laboratories on the specimen of blood[11] or the divergent reports by serologists on different specimens of blood from the same patient, conveyed the hasty and immature impression to the physicians at large that the Wassermann reaction was not only worthless but that it was misleading. Yet the irresistible conclusion that stands out in bold relief, after the study of these reports, is that none of the authors,[11] except one,[12] questioned the specificity of the reaction as far as it relates to syphilis. Furthermore as the test is being perfected the diseases that were supposed in the past to have been associated with a positive complement-fixation test, one by one have been crossed off the list. The last disease to lose this distinction is leprosy.[13] Yaws remains the only other entity besides syphilis where the blood persistently exhibits inhibition of hemolysis. The belief that the cholesterinized antigen is the underlying cause of falsely positive reports has been principally based upon lack of correlation between the clinical findings and the negative history on one hand and the positive serologic findings on the other. At this point of the discussion, it would not be irrelevant to point out that the attitude of the clinician who assumes that he is infallible in the matter of the diagnosis of syphilis is not always supported by the pathologist. Cases are on record, where neither histories nor the physical examinations have alluded to the possibility of the presence of syphilis, and yet, Treponema pallidum has been demonstrated histologically to be present in the tissues.[14] The fear that the contradictory reports on the same specimen of blood by different serologists or by the same serologists on repeated specimens from one patient, and which constitutes one more indictment against the reliability of the reaction, is not founded upon fact. It is well to note that the reports in themselves are consistent.[11] The laboratory rendering a positive report on a specimen of blood invariably rendered the same report on the repeated tests carried out on the same individual. This holds true likewise when a negative result was rendered.

Only in the matter of a doubtful or 1+ reaction have the reports been conflicting. The latter inconsistencies are more apparent than real. It is common knowledge that during the phenomenon classed as latent syphilis,

in the course of the disease a patient may harbor the Treponema pallidum in the spleen, the marrow, the lymph nodes, semen, the testicles, and the material capable of transmitting the disease when used for inoculating purposes.[15] Nevertheless, the antibody-like substance that determines the fixation of the erythrocytes may be so small in amount in the circulating blood of the patient that the intensity of the reaction may vary and the report may read 1+ with cholesterinized antigen and at the same time be reported negative with the alcoholic extract. The factor most commonly responsible for the occurrence of the latter phase is the antigen employed. Hence, the clinical interpretation of the value of a positive or negative test, regardless of its intensity, is largely a matter of one's knowledge of the value and significance of the technic used.

The outline of the Wassermann test,[16] as it is done under the direction of B. L. Crawford, Clinical Laboratory, Jefferson Hospital, is appended to avoid ambiguity.

The antisheep-hemolytic system is used, and the technic consists in using inactivated serum in 0.2 c.c. amounts. A solution of complement of constant strength is used, against which the amboceptor is titrated every day, and two units of amboceptor are used in the test. Two antigens are used, cholesterinized and alcoholic extract of syphilitic liver; however, the specific antigen is not used with all the serums tested each time, but both antigens are used with a sufficient number of serums each time to have a control on the cholesterinized antigen. The first incubation of the mixture of serum, antigen and complement is done in a water-bath at 37° C. for one hour. Then 1 c.c. of a 1:40 dilution of washed sheep cells and two units of amboceptor are added separately and incubated for one hour. At the end of this time, readings are taken if the negative controls show complete hemolysis and positive controls show complete fixation.

As a matter of precaution, the tubes are put in the ice box overnight and a final reading is taken the following morning.

SUMMARY

The purpose of this paper is to emphasize the importance of a 1+ Wassermann reaction with the cholesterinized antigen, but not to maintain that this test *per se* is diagnostic of syphilis.

Experimental and postmortem evidence is not lacking that a patient may be afflicted with syphilis; that he may be a source for transmission of the disease and yet not manifest the disease clinically.

To have adopted the dominating view that there is no need to attach significance to a partial cholesterinized serologic reaction, the patients previously mentioned, one suffering from syphilis of a kidney, another having latent syphilis, and the third, obviously having asymptomatic neurosyphilis, would have been assured, in view of the fact that their physical examinations and histories were negative, that syphilis was not the cause of their ailments and left to the ravages of the disease.

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POLLEN SENSITIZATION: A PRELIMINARY REPORT*

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THOSE who are engaged in the prophylactic treatment of hay-fever are well aware of the fact that satisfactory results are not sufficiently constant to justify one in stating that the treatment of this condition is on a stable basis. While complete freedom from symptoms is often obtained, there are large numbers of individuals whose improvement is moderate or slight and, since there is no clinical criterion by which it may be foretold into which class a given person may fall, the conscientious practitioner must be guarded in the amount of relief promised.

The difficulty in obtaining satisfactory clinical results may be due to, (1) Unsatisfactory material used in treatment, (2) Unsatisfactory method of administration of treatment, (3) Insufficient treatment, or (4) The conception of the mechanism of the condition may be fundamentally unsound. The work here presented deals only with the first and last of these possibilities.

The most generally accepted opinion of the underlying mechanism of hay-fever seems to be that it is an anaphylactic phenomenon—a response to the introduction of a pollen protein to which the individual has, in some manner, become sensitized. The arguments which have been advanced against this theory are (1) that anaphylaxis, as we know it in the experimental

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animal, is not known to exist naturally, whereas hay-fever is very common, affecting possibly 1 per cent of the population[1]; (2) anaphylactic animals may be rendered refractory to subsequent introduction of the specific protein by a single injection of the protein. This has not been found possible in hay-fever. (3) The anaphylactic state may be passively transferred to normal animals while the very few reports of such transference in hay-fever are probably open to question.

STANDARDIZATION OF POLLEN EXTRACTS

In attempting to secure some information relative to the question of of anaphylaxis as the possible basis of hay-fever, it was recognized that in work extending over a period of months requiring the use of more than one pollen extract, some definite knowledge of the actual content of active substance in these extracts must be obtained and some means of comparing the value of these extracts in terms of active substance must be had. The first work done, therefore, was an investigation into the question of standardization of extracts.

The clinical criterion of activity in general use has been the skin reaction of the hay-fever patient. An inherent difficulty, generally recognized, is the wide variation in reaction of the skin of different individuals which makes the method wholly inadequate for quantitative work. It is also a possibility which has, apparently, not always been kept in mind, that an individual who has been treated with a pollen extract may give a reaction to the protein of the extract used in the test which may have no relation to the reaction due to the *active substance* of the extract. The skin reaction may, in this event, be a composite of reactions to two or more substances which would entirely invalidate it as a means of determining the amount of *active substance* in the extract. Again, it must be kept in mind that both the scratch method and the intradermal injection are in use and that reactions obtained by the two methods may not be quantitatively comparable.

To avoid these difficulties this investigation was carried out entirely upon myself as I am a typical ragweed-sensitive individual. Believing that the typical nasal reaction to pollen might be used as a satisfactory standard for measuring activity of extracts, these were introduced into the nose in increasing amounts until sneezing was produced. It was found that when this was done in the part of the year when there was no ragweed pollination and when there was no nasal irritation from other causes, the minimal amount of a given extract which would produce sneezing was exceedingly constant. Sneezing would begin within five minutes and, if 0.1 mil of a given solution was required on a given day the same amount would produce the same reaction on any other day and less than this amount would not elicit any reaction on any day. Because of its dependability and because it made it possible to get away from the questionable skin reaction, the intranasal reaction was adopted as the index of activity.

The pollen extracts used included ragweed extracts from each of the manufacturers marketing these products and two extracts made by myself. The two latter were made by the method proposed by Coca.[2]

There are three methods of standardization now in use: (1) A given amount of pollen is extracted with a given volume of extracting fluid and the resulting extract designated as a given dilution of whole pollen or as containing so many pollen units. (2) The extract is Kjeldahled and the nitrogen content used as the basis of calculation. (3) By complement fixation.

The fallacies inherent in the first method are apparent. First, if given amounts of pollen are extracted with fluids of different composition, the solubility of the active substance in the different fluids may produce extracts of widely differing activity and these extracts would not be comparable. Secondly, extracts made by the same method may vary in content of active substance. Thus, the two extracts made by myself, using the same extracting fluid and technic throughout with different batches of pollen (but secured from the same source) yielded solutions one of which was 62½ times as potent as the other.

Standardization by nitrogen content has for some time been considered unsatisfactory because of the belief that the antigenic nitrogen compounds form only a part of the total protein present and the nitrogen determination makes no distinction between antigenically active and inert protein. Previous investigators[3] have concluded that this is an inadequate method, while Grove and Coca[4] have recently stated that extracts digested with trypsin and dialyzed are still active as formerly.

We have made nitrogen determinations on eight ragweed extracts. Table I shows the nitrogen content as compared with the minimal amounts required to produce the intranasal and intradermal reactions.

It is readily seen that the nitrogen content has no quantitative relationship to the activity of the solution. Whereas the largest nitrogen content is only 18 times the smallest, the largest amount required to produce sneezing is 2,500 times the smallest. Also the solution containing the most nitrogen is only one-half as active as the one containing the least.

If it be insisted that the skin reaction be taken as the index of activity it is again seen that there is no relationship. The solution with the least nitrogen content is eight times as active as that containing the most, while the dilution of the most active extract producing a reaction is 6,400 times as great as the dilution of the least active producing the same reaction. Extracts C1 and C2, made in the same way, show a wide variation in nitrogen content and activity both in nose and skin, C2 containing little more than one-half the amount of nitrogen in C1 but reacting 62½ times as well in the nose and 128 times as well intradermally.

TABLE I

EXTRACT	MG. N. PER MIL	INTRANASAL UNIT	INTRADERMAL UNIT
A^1	1.26	.1 mil	1-1,000
A^2	.28	.1	1-4,000
C^1	.98	.005	1-20,000
C^2	.56	.00008	1-2,560,000
L	.42	.005	1-8,000
M	.07	.05	1-8,000
P	.14	.2	1-400
S	.70	.00025	1-1,280,000

Table I also illustrates the relative parallelism of the intranasal and intradermal reactions; that is, those most active intranasally are also most active in the skin and vice versa. This parallelism in one who has had treatment with pollen extracts indicates that the possibility previously mentioned of reactions in the treated patient to protein which may have no relation to the true reaction of the hay-fever patient, is probably not of any moment.

We were able to repeat the work of Grove and Coca[4] and, by digestion with trypsin and dialysis against distilled water, secured an extract which no longer gave a biuret reaction. This digested extract reacted intranasally and intradermally in the same dilutions as it did prior to digestion.

The method of standardization by complement fixation first advocated by Clock[5] has been believed to offer a means of determining the antigenic protein as distinguished from that which is antigenically inert. It is possible to inject animals with pollen extracts and produce antibodies against them. We have found, too, that the serum of ragweed-sensitive persons contains a substance which binds complement in the presence of ragweed pollen. In tests done using my serum it was found that 0.002 mil gave complete fixation of complement. Tests were then done using 0.2 mil of a 1-10 dilution (10 units) of serum and the results are shown in Table II.

TABLE II

EXTRACT	MG. N. PER MIL	UNIT FOR COMP. FIX.	INTRANASAL UNIT
A^2	.28	.02 mil	.1 mil
C^1	.98	anticomp.	.005
C^2	.56	anticomp.	.00008
L	.42	.05	.005
M	.07	.2	.05
P	.14	.02	.2
S	.70	.002	.00025

It will be seen that the two extracts made by Coca's method were anticomplementary and complement fixation as a method of standardization was, with them, impossible. The extract with the least nitrogen content was the weakest antigenically while that with the highest amount of nitrogen was the most potent. Between these extremes the parallelism was not regular. On the other hand, a comparison of the binding power with the intranasal activity shows only a general approximation. Extract P which has ten times the antigenic value of Extract M is only one-fourth as active in the nose.

Complement-fixation tests done on the sera of treated and untreated hay-fever cases lead us to believe that the complement-binding substance is produced as a response to the introduction of some one or more of the pollen proteins and not necessarily to the active substance itself. Three individuals who received from 20 to 30 injections of pollen extract during the past season were capable of binding complement with 0.002, 0.002 and 0.001 mil of serum, respectively, while of five ragweed-sensitive individuals who have never had any treatment, the amounts of serum required varied from 0.1 to 0.3 mil. This small number will not permit of any hard and fast conclusions but we are inclined to believe that the complement-binding substance is found in the

serum of the untreated as a result of absorption of the protein through the mucosa of the upper respiratory tract while the increased amount in the sera of treated persons is due to the repeated injection of the protein.

If the foregoing assumption may be questioned we feel that we have more dependable data in the results of tests done with digested extracts. Extract L whose antigenic unit was 0.05 mil was antigenically inert after digestion, although its activity intranasally and intradermally was unchanged. Guinea pigs which had been injected with Extract C2 (which was anticomplementary and could not be used for fixation tests) responded with the production of an antibody which fixed complement in the presence of other ragweed antigens. These sera gave no binding with the digested extracts. Extracts L and C2 were mixed with equal parts of my serum and injected intradermally into the forearm of a nonsensitive individual.[6] Typical reactions were obtained. These extracts, after digestion, although no longer capable of serving as antigens in complement-fixation tests, produced the same reaction, quantitatively, in the arm of this nonsensitive individual.

Since extracts which have been digested and can no longer serve as antigen in complement-fixation tests, are as active intranasally and intradermally as before, we believe the conclusion is justified that complement fixation as applied to pollen extracts is only another method of determining the protein in the extract and, while it may serve to distinguish some proteins from others, it is, as a means of estimating the amount of active substance, entirely valueless.

THE NATURE OF THE ACTIVE SUBSTANCE

The work presented in the foregoing statement warrants the conclusion, we believe, that the active substance in pollen is not protein. Grove and Coca[4] suggested that it resembled an enzyme. We have shaken ragweed extract four hours in a mechanical shaker and found its activity unchanged. It has been frozen solid for six days and lost none of its activity. Boiling for ten minutes and heating it in the autoclave at ten pounds for ten minutes did not affect it. The evidence does not support the assumption that the active substance is an enzyme.

The work done by Heidelberger and Avery[7] in which they found in pneumococcus substance what they believe to be a polysaccharide capable of producing precipitates with immune serum, led us to attempt to correlate the activity of pollen extracts with their glucoside content. This work has only been begun but, thus far, we have found that the Molisch reaction does not parallel the activity of the extract and reduction of Fehling's solution does not seem to be increased after hydrolysis. This work is still in progress.

EXPERIMENTS BEARING ON ANAPHYLAXIS

In an attempt to produce active anaphylaxis in guinea pigs Extract C2 (containing 0.56 mg. nitrogen per mil) was injected intraperitoneally into six pigs whose weights were from 250 to 320 grams. Three pigs received a single injection of 2 mils for each 250 grams body weight. Three received one mil per 250 grams body weight and forty-eight hours later a second

injection of the same amount. Five days after the first injection one pig which had received the divided doses was found dead. Two days later one receiving a single large dose died. On the tenth day three were found dead and on the eleventh the last succumbed. No evidence of illness was noted except a slow but constant loss of weight, which, in no instance, exceeded 15 per cent. In each instance the animals were found dead without any opportunity to observe any agonal symptoms. Autopsies revealed no gross lesions. Since this was the extract which proved to be 62½ times as potent as the next most active extract, it was thought that death was probably due to excessive dosage.

Six pigs weighing from 250 to 350 grams received one mil of Extract C2 intraperitoneally and fourteen days later were given the same amount intracardiacally. No symptoms even remotely suggestive of anaphylaxis were observed. Three pigs received 5 mils each of my serum intraperitoneally and forty-eight hours later were given one mil Extract C2 into the heart. The pigs were apparently entirely unaffected. Two rabbits were injected intracardiacally with a freshly made mixture of 2 mils of Extract C2 and 5 mils of my serum. They showed no symptoms of any kind. Two rabbits received similar mixtures in the veins of the ear with no effect.

The pigs which had received the sensitizing and shocking doses of pollen without effect were bled and their serum separated. These sera, when mixed with Extract C2 and injected intradermally into the forearm of a nonsensitive individual, produced a typical reaction.

Although in this small series of animals we were unable to elicit any evidence of anaphylaxis, we would not conclude that anaphylactic reactions cannot be secured with pollen extracts. Alexander,[8] working with extracts of rye pollen, reported typical reactions. We believe that the difference in results is due to the fact that he was working with a solution containing 2.01 mg. of nitrogen per mil while ours contained only 0.56 mg. We believe that anaphylactic reactions may be secured by the use of extracts rich in protein and poor in active substance but that, like complement fixation, these reactions are reactions to the protein of the extract and have no relation to the active substance.

SUMMARY

1. The reaction of the nasal mucosa of a ragweed-sensitive individual may serve as a reliable criterion for the determination of the amount of active substance in ragweed pollen extracts.

2. None of the methods of standardization of pollen extracts now in general use are of value.

3. Solutions made by the same technic may differ widely in content of active substance.

4. The active substance is not protein.

5. The complement-binding substance is apparently increased by treatment.

6. Complement fixation, as applied to pollen standardization, is only another method of estimating certain protein constituents of pollen.

7. No evidence of sensitization of skin to protein, as distinguished from active substance, in treated cases was found.

8. Experimental evidence suggests that hay-fever is not an anaphylactic phenomenon.

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IS PATHOLOGIC NOMENCLATURE EITHER SCIENTIFIC OR SENSIBLE?*

By O. J. West,† M.D., Seattle, Wash.

RECENTLY, before our local Medical Society, an orthopedic surgeon read a very excellent paper on "Bone Tumors."

In the course of this paper he presented a classification of these growths, entirely of his own devising, extenuating this by explaining that recent textbooks differ so widely in their terminology that the more one reads, the more he becomes confused.

I, being a pathologist, even I may say, being an old-fashioned pathologist; a pathologist of the period far back in the good old days when the embryonic layers were epi-, meso-, and hypo-blast, the epiblast developing into pavement epithelium and nervous tissue, the mesoblast giving rise to the framework structures of the body, the hypoblast to glandular epithelium, each layer attending strictly to its own work, never encroaching on the field of the others; when malignancy of epithelium was always carcinoma and malignancy of mesoblastic structures always sarcoma. As I have said, being an old-fashioned pathologist trained in this atmosphere of exactness, I would have resented my colleague's intimation that pathologic terminology is lax and erratic, had I not recently made various book reviews for our local medical journal.

These reviews were of textbooks on pathology and tumor diagnosis and, perusing them, one after another, one needs must be impressed with the faulty and unscientific nomenclature in vogue.

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†Pathologist, Providence Hospital.

For the purpose of specific data, I visited the medical library and briefly sketched through several of these, presumably, authoritative works. In them I found no less than 12 *malignant* growths described under names which in no way indicated their malignancy, nor whether of epithelial or connective tissue origin—misnomers, such as cylindroma and psammoma—a tumor of cylinders and a tumor of sand! I ask you, is that scientific or even common sense? Are the cylinders and the sand of such importance that they should command the terminology, to exclusion of the malignancy resident within these growths?

Nor does the perniciousness end with this. Just so surely as a tumor is named from some *characteristic—not* the tissue of its origin, just that surely will inaccuracy and confusion arise.

This is exemplified in both the misnomers I have mentioned.

In Prof. Ewing's work on, "Neoplastic Diseases," page 294, he describes cylindroma among the endotheliomata, on page 705, he says: "Under the term cylindroma has been described a rather frequent form of adenocarcinoma of the salivary glands."

Wood's revision of Delafield and Prudden says: "Some of these growths are carcinomata." What the others are he does not state, but, already, we have the same growth, an endothelioma, an adenocarcinoma, a carcinoma and so named that it indicates neither.

Psammoma fares no better. Ewing, page 296, classes it with the endotheliomata and on page 424, writing of psammoma of spinal dura says: "The structure presents * * * a cellular and vascular form of sarcoma." Other authoritative works say they are composed of either fibrous, sarcomatous, or endotheliomatous tissue. In other words a psammoma may be a benign fibroma, a malignant sarcoma or a semimalignant endothelioma.

Again, is there either science or sense in such terminology? Striving for clarity only turbidity has resulted. Nor is this roiling of the pathologic waters necessary. If this sand is truly of importance it can be incorporated into the nomenclature without outraging pathologic tenets. Call it a psammomatous fibroma, or sarcoma, or endothelioma, as the case may be.

We also find various neoplasms named from the *organ* wherein they originate, such as thymoma, hepatoma, hypernephroma. To justify such terminology these should be an autonomous growth of *all* the tissues of these organs in orderly arrangement. Instead, thymoma is really a lymphosarcoma, hepatoma is adenoma or carcinoma and hypernephroma, a tumor of *adrenal* tissue, sometimes orderly, often malignant.

Is this scientific?

Even in the nomenclature of pathologic tissue-changes, confusion and inaccuracy appear in ever-increasing amounts.

Seemingly this arises mainly from efforts diagnostically to connect the histologic findings and the clinical symptoms. In other words, having certain symptoms, there always must appear certain characteristic histologic changes.

I do not believe that any amount of study *ever* will accomplish this, for the insurmountable reason that different individuals will not react uni-

formly to the same tissue change, more than they will react in the same way to such patent things as an infection process, or drugs, or even light and heat.

Who among you would stake his pathologic reputation that a case showing frank fetal adenoma of the thyroid would have had thyrotoxic symptoms?

The pathologic changes are what they are, let the symptoms be what they will, and so soon as we permit the clinician, whether internist or surgeon, to tamper with our terminology, just that soon will confusion and obscurity obtain.

Some surgeons refuse to consider a growth malignant unless it customarily metastasizes, be the cells never so anaplastic and atypical. The influence of this idea already is found in our nomenclature, and, should the pathologist accept it, further confusion will arise.

I believe that only by adhering strictly to structural histology will pathologic nomenclature be fitted to a science.

I am fully cognizant that some histologic carcinomata and sarcomata very rarely metastasize. Does that preclude them being designated as carcinomata and sarcomata? Is a nonmetastasizing carcinoma so *vast* an anomaly when compared with other disease-conditions as we know them!

Since it is recorded that man has died of the itch and has recovered from carcinomatosis, who would dare forecast with absolute certainty how this human body will react to a given condition or what will be the conduct of a given neoplasm that is implanted in that body! The only thing that may be absolutely certain about a tumor is its histologic structure, and science can be built only on certainties. For this reason I decry the tendency to dominate pathologic nomenclature by *characteristics* of tumors, or *clinical symptoms* of pathologic changes. Our terminology must be based upon *fundamentals* if we would hold it free from obscurity, and it is surprising to what few basic elements microscopic histology can be reduced if we hold fast only to proved facts and ignore theory and surmise.

I have no doubt many of you would be extremely skeptical were I to state that there are but two *general* types of tissues in this wonderful human body of ours, and yet I can assure you that it so closely approximates actuality that, for years, it has been of the greatest aid to me in pathologic study.

By "two general types" of tissue I mean; first, the tissue which *secretes* —the *functional* tissue of an organ, and second, the *framework* tissues that hold this functional portion in place.

Tersely, secreting tissue and framework tissue.

Concisely: epithelium, and the tissues of mesoblastic origin. Of the epithelium there are two varieties, but malignancy of *either* develops into carcinoma. Of the mesoblastic structures there are several, fibrous, muscle, bone, fat, cartilage, lymphoid tissue—but malignancy of each develops into sarcoma. Thus we have but two types of malignancy, carcinoma and sarcoma.

In apposition, from a single textbook I glean an impressive array of: acanthoma, adamantinoma, astrocytoma, chloroma, chorioma, cylindroma, folliculoma, hepatoma, hypernephroma, myeloma, psammoma, splenoma, thymoma, etc., yet all of these are really either carcinoma or sarcoma.

Are we being more exact in return for the increased burden pathologic literature must bear? We are not. What accuracy is there in terming a malignant growth a tumor of thorns, or stars, or chlorophyl, or follicles or sand?

While the pathologist may be trained to knowledge of the meaning and significance of these terms, it should be borne in mind that the field worker, the clinician, is the terminal of all our pathologic labor, and what is he to do with such?

It is all so needless! If a growth is a cancer, let it be so called, if it is sarcoma, so name it, and add to these any enlightening terminology desired, but let them be called carcinoma or sarcoma, first, last, and all the time.

Granted that they vary in clinical course and prognostic significance, is there any other pathologic state that does not, also, vary? Can you make pathology any more exact than are the diseases of which it is a study?

And does the naming of malignant growths after thorns and cylinders and sand, aid in the matter?

Criticism which suggests no remedy is better left unsaid, and the object of this paper is to preface the suggestion that a committee be appointed to draft a sane and sensible outline of nomenclature to be followed by the members of this Society.

I take the liberty of voicing my own views, not that they may be adopted, but that they may stimulate discussion of this subject. Personally, I feel that this outline should be based upon the normal tissues wherein the pathologic changes occur and that all further refinements of descriptive terminology be compassed by means of qualification of these terms; that malignancy of epithelium be designated as carcinoma; malignancy of mesoblastic structures as sarcoma and that the basis of microscopic study of diseased organs be the two types of tissues of which all organs are composed, parenchyma and stroma.

Since these comprise the entire organ it follows that any and all changes must be in one, the other, or both, and we will have parenchymatous, interstitial or diffuse lesions. With this as basis of our terminology, fewer pathologic impossibilities will be present in our literature, such as the very prevalent term, "Parenchymatous Nephritis." If this term is intended to convey the meaning of inflamed parenchyma of the kidney, it is a pathologic impossibility. Having no blood vessels, the parenchyma *cannot* inflame. If there is inflammation the *stroma* must also be involved and we have a diffuse nephritis. If the parenchyma *alone* is involved the change must be degenerative and cannot be inflammatory, therefore a nephritis does not exist. Prof. Wood has suggested that these noninflammatory degenerative changes be termed nephroses, which, to me, seems excellent.

As microscopic histologists, what are we to do with the so-called "precancerous" changes?

Modern textbooks show microphotographs of breast tumors, where the gland acini are widely separated by fibrous tissue, enlarged to 20 times their normal dimension and densely packed with cells which are rapidly multiplying, as shown by the more embryonic character of the cells and pressure-elongated nuclei. (See Ewing's Neoplastic Diseases, 1st Ed., Fig. 184.) Yet

this is called a *precancerous* condition. Presumably, because the cells have not yet broken through the basement membrane and invaded the surrounding tissue.

Is it rational to suppose that the first movement of a malignant gland-cell will be to break through and invade the resistant fibrous stroma? Or is it more reasonable to assume that, as these cells multiply, the resultant cell-mass will follow a law of physics and first progress in the direction of least resistance, which is the *lumen* of the gland? When the lumen is packed to the density of the surrounding tissue, only then would these rapidly proliferating cells invade the stroma.

This cell proliferation in the direction of least resistance is one of the gross diagnostic points of those cancers which are freely open to observation, skin cancer.

At first these growths are elevated because the proliferating cell-mass finds it easier to extend *upward* toward the surface than *downward* through the dense fibrous tissue. Later this fibrous tissue gives way and the malignant area may be depressed, but, invariably, at the edge, where there is yet firm fibrous tissue, the growth is elevated, giving a diagnostic point of some value.

Frankly, I am suspicious of any gland which has filled its lumen with actively proliferating cells, because this is contrary to nature's normal way. When the epithelium of a normal gland proliferates, cell division is longitudinal and at right angles with the periphery of the gland so that it leaves the new and the old cells shoulder to shoulder so to speak. These cells are like a company of soldiers formed in a circle shoulder to shoulder and facing inward: when typical mitosis occurs it is as if one cleaved a soldier from crown to crotch, each half then developing to a complete individual; were this continuously repeated, the line would become more compact, if there were slight peripheral resistance the circle would enlarge and we would have the equivalent of a cystic gland; when peripheral resistance becomes sufficient the line would "buckle" inward, remaining, however, as a single line, and we have the equivalent of a cystic gland with papillomatous protrusion into the lumen. When atypical mitosis of malignancy occurs, cell division is at right angles to that just described, the new illustrative soldier would then stand *in front* of the old and we soon would have many concentric lines, eventuating in a solid mass—the densely packed gland of malignancy.

One who has observed with what reluctance normal gland-epithelium will begin atypical mitosis and pack the gland-lumen with rapidly multiplying cells surely must attach grave significance to such findings.

We must suppose that such growths are called "precancerous" because they are not yet invasive or metastasizing, but to me, this is no more rational (following the old idea that smallpox is not infectious until the period of eruption) than to say that a case of smallpox is not smallpox but a "prevariolar" state, until the stage of eruption or infectiousness.

I do not believe the condition described is "precancerous." I think it is already cancerous, in all probability a preinvasive and premetastasizing stage of cancer but, nevertheless, carcinoma. I believe they should be desig-

nated as *early* carcinoma, but I also believe our reports should further state that the condition is probably as yet neither invasive nor metastasizing, in order that the patient may be subjected to only the necessary degree of mutilation.

In conclusion let me say that this paper was written because of the numerous criticisms of pathologic nomenclature which have been expressed in my presence by active workers in the medical field; and because I believe it lies within the province and influence of this Society to give a trend that is both scientific and sensible to the terminology used by its members.

Announcement

The Ninth Annual Clinical Session of the American Congress on Internal Medicine will be held in Washington, D. C., March 9-14, 1925.

Washington clinicians and investigators of attainment will devote the entire session to amphitheatre and group clinics, ward "rounds," laboratory conferences, lectures, demonstrations of special apparatus and methods, and the exhibition of unusual scientific collections. Civilian and governmental services are united in the aim to make the week useful and memorable.

Practitioners and laboratory workers interested in the progress of scientific, clinical and research medicine are invited to take advantage of the opportunities afforded by this session.

Address enquiries to the Secretary-General.

Wm. Gerry Morgan, Pres.
Washington, D. C.

Frank Smithies, Sec'y-Gen'l.,
1002 N. Dearborn St.,
Chicago, Ill.

THE EFFECT OF VARIOUS ANESTHETICS UPON THE STRENGTH OF UTERINE CONTRACTIONS

By M. Pierce Rucker, M.D., Richmond, Va.

WHEN a hollow viscus contracts in an energetic manner pain is a result. The uterus is no exception to this rule. In no other organ, however, do we associate the idea of force with such cramps. The intestines or the gall bladder may contract as painfully as the uterus, but we do not get the impression of forcefulness that we do in the presence of labor pains. From time immemorial force and pain have been so inseparably associated with parturitional contractions that we unconsciously use the two terms interchangeably.

The force of labor pains has been variously estimated at from 4 to 577 pounds.[1] This wide variation was due to the different methods used, such as measuring the force required to rupture the amniotic membranes outside the body, the pull necessary to deliver the infant with forceps, etc. In 1866 an instrument designed by Carcassonne[2] to measure the contractions of the uterus in parturition was presented at a meeting of the French National Academy of Medicine. There was no description of the instrument which was called the "metrodynamomètre," nor can I find any record of the work done with the instrument. When twelve years later Poulet[3] presented his instrument, which he called the "tocograph," before the Surgical Society of Paris a committee composed of MM. Guéniot, Lucas-Championnière and Polaillon was appointed to look into the merits of the instrument. Polaillon gives a description of the instrument in his report[4] and states that the only other similar work is that of "Schartz of Rastok." The tocograph consists essentially of two mercury manometers, one connected to a rubber bulb that is placed within the uterus and the other to a similar bulb that is placed in the rectum above the fetal head. The manometers were provided with floats and writing points, and in this way records were made upon Ludwig's kymograph of the action of the uterus and of the abdominal muscles. At the conclusion of his report M. Polaillon moved (and it was adopted):

1. "De remercier M. Poulet de son travail.

2. "De déposer sa mémoire aux archives.

3. "De publir, dans nos mémoires, la figure indiquant les courbes des pressions utérines et musculaires."

Nevertheless in the published memoirs there are no uterine tracings illlustrated.

In 1872 Schatz[5] published his first work upon uterine contractions, and his curves and figures are still referred to in American obstetrical literature.

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Schatz found an intrauterine pressure of 20 mm. of Hg. between pains, and of from 80 to 250 mm. of Hg. at the acme of contractions. Polaillon's[6] work in 1880 is in some respects even more interesting, but has attracted little notice in this country. He used an intrauterine rubber bulb and a mercury manometer for measuring the force of uterine contractions as did Schatz and Poulet, but for recording the contractions he used a Marey tambour. He

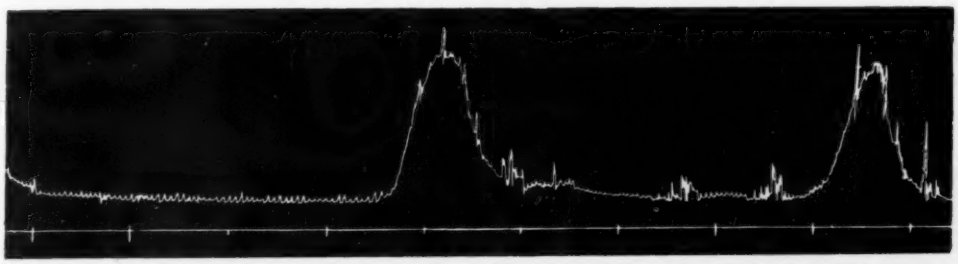

Fig. 1.—Internal hysterogram showing first stage pains with small reflex contractions of the abdominal muscles at the top of the contractions and respiratory movements between contractions. The timer in this and all subsequent figures marks minutes.

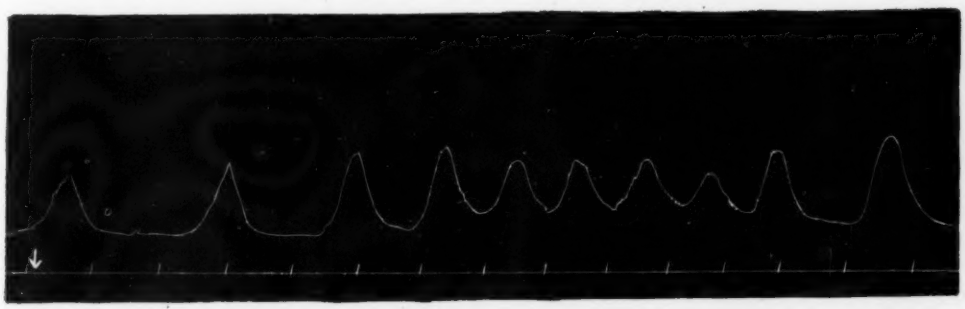

Fig. 2.—Internal hysterogram from a patient whose labor was induced prematurely and who was given 2 minims at the point indicated by the arrow. Note that there was very little effect, but that the uterus did not relax completely for nine minutes.

Fig. 3.—This patient was at term and was given 2 minims of pituitrin at the point indicated just at the end of the second contraction in the record. In this instance there was a marked increase in intrauterine pressure that did not subside for 28 minutes.

divides the accessory uterine movements into intrinsic and extrinsic. Among the latter may be mentioned respiration and sighing, crying, sneezing, muscular efforts in moving the head or the body, straining, micturition, defecation, pulsations of the maternal arteries, and pressure of the hand on the abdomen. The intrinsic accessory movements are the fetal movements, which

are very irregular. Polaillon marked on the curves of uterine contractions the exact point at which the patient felt pain and when she ceased to feel the pain. He found that this occurred constantly when the curve reached a height of 12.25 mm. of Hg. and ceased when it fell below 10.45 mm. of Hg. In a fatal case he weighed the uterus, and by calculating the amount of work that had been done from his records, estimated that a gram of uterine muscle was capable of putting forth 178 grams of energy which is small compared to the figures (1087 grams) given for striated muscle. In 1898 Hensen[11] investigated the action of morphine and ether upon uterine contractions, using Schatz's apparatus. Schatz, Poulet and Polaillon all used small rubber bulbs of from 70 to 80 c.c. capacity, that were placed within the uterus between

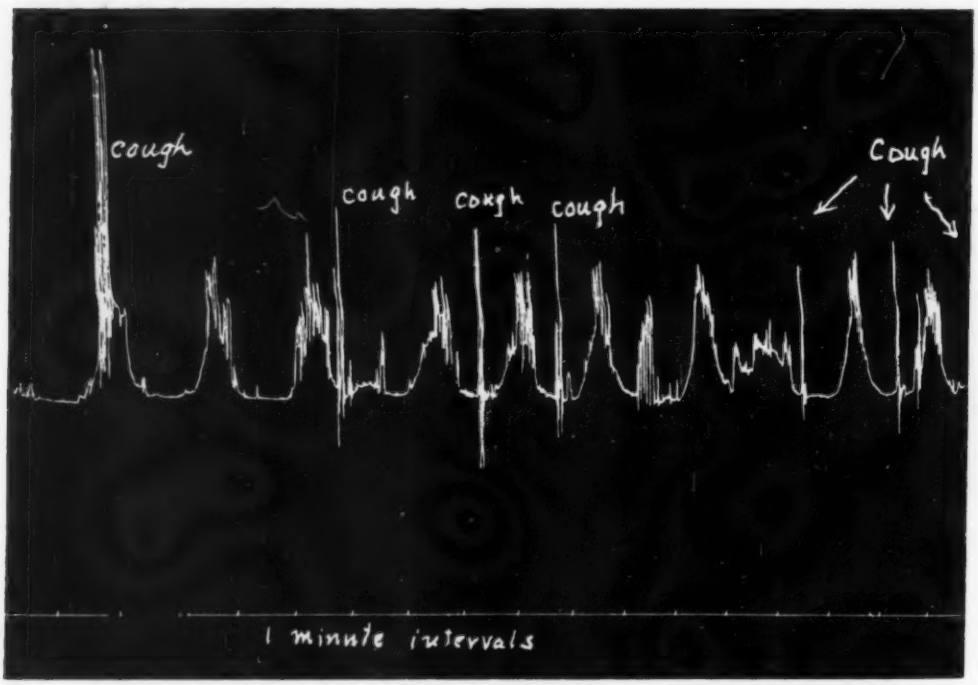

Fig. 4.—Internal hysterogram showing first stage pains with superimposed involuntary contractions of the abdominal muscles. Between the pains are shown the effects of coughing (sharp straight lines) and of respiration, (the tiny waves of 1 or 2 mm. in height).

the membranes and the uterine wall for the purpose of registering the variations in intrauterine pressure. Several years ago, it occurred to me[7, 8] that I might in the same manner make use of a Voorhees bag that had been placed within the cervix, without detriment to the patient. The stem of the bag was connected with a mercury manometer by means of a thick wall rubber tube. The distal arm of the manometer was fitted with a float that carried a writing point.

Fig. 1 is from a tracing of normal first stage contractions obtained in this manner. It is very similar to Schatz's and Polaillon's. It shows an intrauterine pressure of 10 mm. of Hg., while at the height of the contraction the pressure reaches 66 mm. of Hg. You will notice that the respiratory

movements cause a variation of several mm. of Hg. in intrauterine pressure. Figs. 2 and 3 show the importance of accurately measuring the variations in intrauterine pressure when studying uterine contractions. It is commonly stated that pituitary extract augments normal uterine contractions, but does not cause tetanic contractions. What happened in these two instances, however, was that after doses of two minims of pituitrin the uterus went into incomplete tetanus with superimposed waves of contraction, which to the palpating hand on the patient's abdomen would feel like normal contractions with periods of relaxation in between. Fig. 4 shows the effect of coughing and Fig. 5 the effect of vomiting and of straining in an effort to void. It is very evident that almost any use of the abdominal muscles is attended by a greater intrauterine pressure than is a contraction of the uterus, but for a much shorter time.

This method of internal hysterography is especially well suited to the study of the effect of various anesthetics upon the strength and frequency

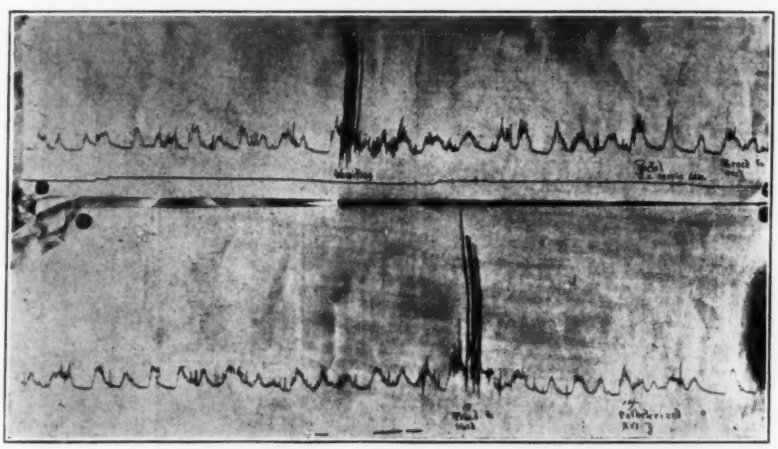

Fig. 5.—Internal hysterogram showing the effect of efforts at voiding and also of vomiting.

of uterine contractions. Chloroform, since the discovery of its anesthetic properties by Sir James Y. Simpson, has been considered, certainly until very recently, the obstetric anesthesia *par excellence.* Sir James Y. Simpson[9] considered it superior to ether for the following reasons:

1. "A greatly less quantity of chloroform than of ether is requisite to produce the anesthetic effect; usually from 100 to 130 drops of chloroform only, being sufficient, and with some patients less. I have seen a strong person rendered completely insensible by six or seven inspirations of thirty drops of the liquid.

2. "Its action is much more rapid and complete, and generally more persistent. I have almost always seen from ten to twenty full inspirations suffice. Hence the time of the surgeon is saved and the preliminary stage of excitement, which pertains to all narcotizing agents, being curtailed, or indeed practically abolished, the patient has not the same degree or tendency to exhilaration and talking.

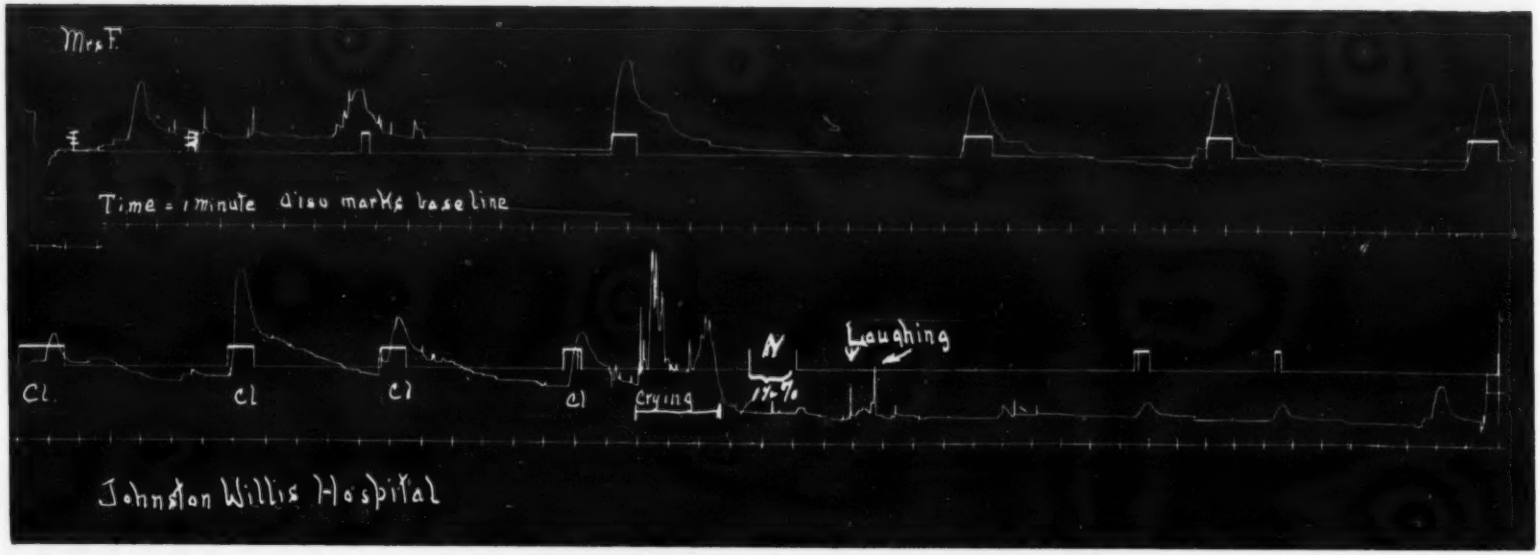

Fig. 6.—Internal hysterogram showing the effect of chloroform given *à la reine*. At the points marked "Cl" chloroform was given in sufficient quantities to somewhat ease patient's pains and to make her hysterical. One and one-half per cent novocaine (35 c.c.) was given sacrally at the place marked "N." The line between the timer and the writing point of the manometer (above it toward the end of the record) is made by an electric magnet that is controlled by push button in the patient's hand, so that the patient can indicate when she feels pain. Note the last contraction on the record is painless.

3. "Most of those who know from previous experience the sensations produced by ether inhalation and who have subsequently breathed the chloroform, have strongly declared the inhalation and influence of chloroform to be far more agreeable and pleasant than those of ether.

4. "I believe that considering the small quantity requisite as compared with ether the use of chloroform will be less expensive than that of ether.

5. "Its perfume is not unpleasant but the reverse, and the odor of it does not remain for any length of time obstinately attached to the clothes of the attendant, or exhaling in a disagreeable form from the lungs of the patient, as so generally happens with sulphuric ether.

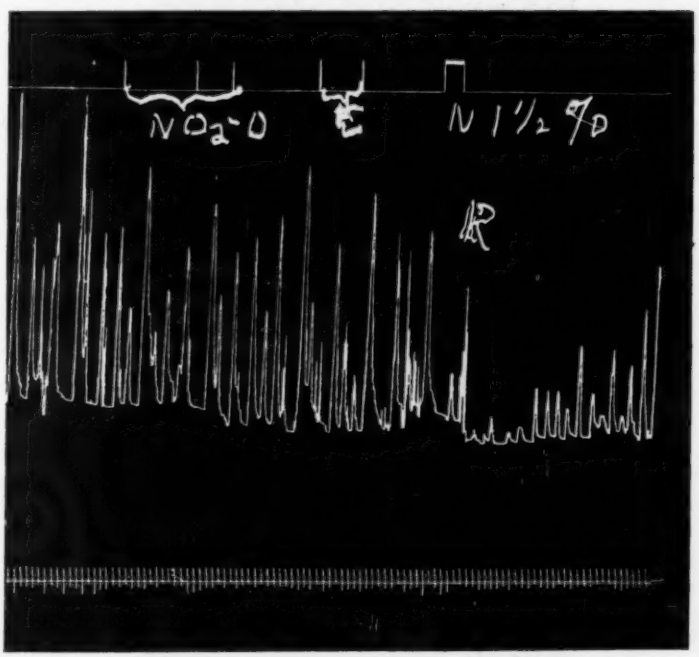

Fig. 7.—Internal hysterogram showing the effect of (1) nitrous oxide-oxygen; (2) ether given *à la reine*. In neither instance was there enough anesthetic given to either relax the patient or to render her unconscious. Note that there was some effect upon the strength and frequency of contractions after the nitrous oxide-oxygen was begun and a step-like rise as soon as it was stopped. The effect of ether was more marked. Later the patient was given 35 c.c. of 1½ per cent novocaine solution with 5 minims of adrenalin solution into the sacral canal. The pains stopped entirely for 11 minutes and when they again started were only one-third their former force. "R" indicates a rectal examination.

6. "Being required in much less quantity, it is much more portable and transmissible than sulphuric ether.

7. "No special kind of inhaler or instrument is necessary for its exhibition."

Even today there is much difference of opinion among obstetricians as to the effect of chloroform upon uterine contractions. Vignes[10] says that this is in part due to the little attention that has been paid to the "temperament" of the anesthetized. There is a great difference between a "country" uterus and a "city" uterus, between the uterus encountered in the clinic and the one met in private practice. The method and time of

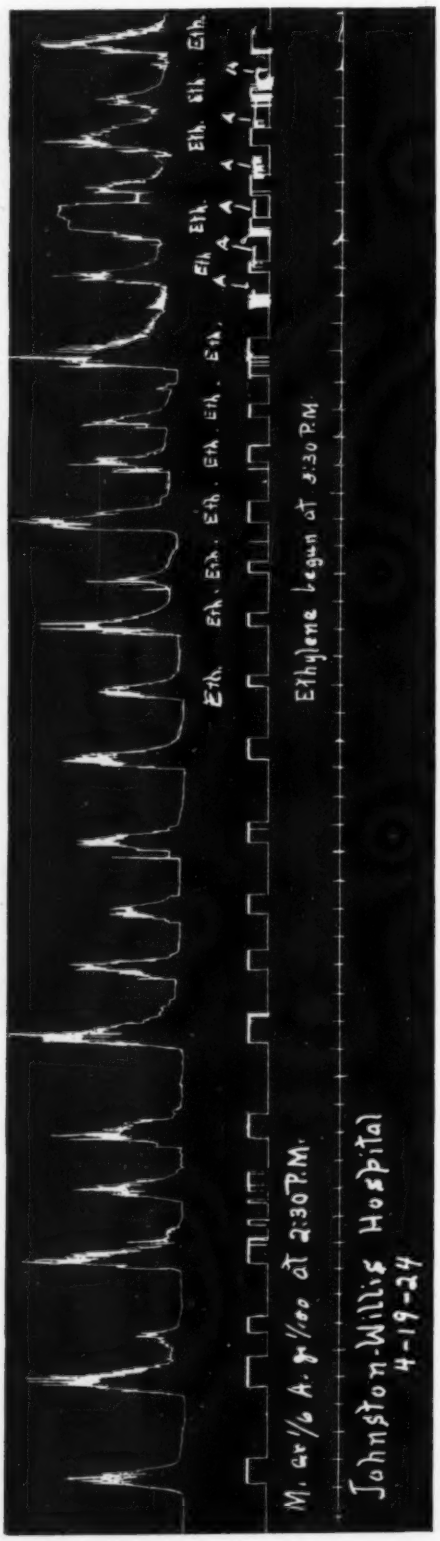

Fig. 8.—Internal hysterogram showing the effect of ethylene administered *à la reine*. The contractions are stronger and last longer. The patient was relieved of sensation of pain.

administration are other important factors. In exceptional cases chloroform can be given *a la reine* for long periods of time without materially influencing the progress of labor. Usually, however, if given too early it seriously interferes with the progress. Fig. 6 shows what is probably the usual effect. There is a diminution in the height of, and a lengthening of the interval between contractions. Fig. 7 shows almost as marked an effect from the administration of ether in the same manner, i.e., *a la reine*. This is in accord with Hensen's[11] findings.

It is quite commonly believed that nitrous oxide-oxygen not only does not diminish the force of uterine contractions, but may even increase it. Danforth and Davis[12] in a recent article say "nitrous oxide is the one thing yet thoroughly tested which will relieve the pain of labor without at the same time diminishing the force and frequency of the uterine contractions, and which may be used for periods up to several hours without appreciable loss in the

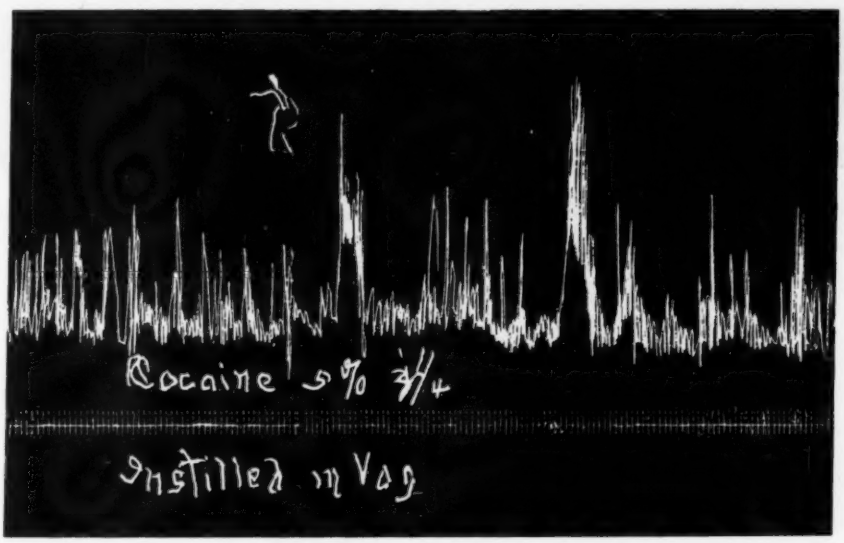

Fig. 9.—Internal hysterogram showing the effect of the application of 5 per cent cocaine solution to the cervix.

force of the uterine contractions." That this is not invariably the case can be seen in Fig. 7. Here there was diminution in both force and frequency of contractions so long as the gas was given, and it was given intermittently at each pain. As soon as the gas was stopped there was a very prompt increase in the strength and the duration of the pains.

Fig. 8 shows the effect of ethylene. When the gas was started at the point indicated the patient seemed to suffer more and became very restless. There was some mental confusion as is shown in the record by her signaling that she had a pain when the uterus was not contracting. The uterine contractions became stronger and lasted longer and were augmented by contractions of the abdominal muscles as indicated by the sharp lines on the summit of the uterine waves. When asked about it afterwards the patient said the gas gave her the greatest relief, that while she knew that she was having pains she did not experience the pain.

Cocaine applied to the cervix has been of distinct value to me in introducing bags, especially in primiparae. Not only does it cause some relaxation and deadens sensation, but it also softens and even dilates the cervix. Fig. 9 is a hysterogram from a patient into whose vagina 15 minims of a 5 per cent cocaine in 1-1000 acriflavine solution was instilled after the bag was placed. Unfortunately, the patient was restless and the record shows a great deal of movement of the abdominal muscles. It is very evident, however, that after the cocaine was applied the uterine contractions were less frequent

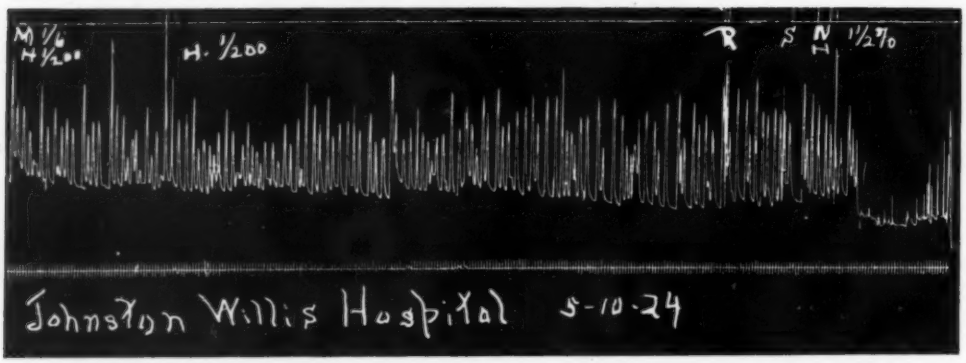

Fig. 10.—Internal hysterogram showing the ordinary effect of sacral anesthesia with novocaine (with 5 m. of adrenalin). Ten minutes after the injection was completed all contractions ceased for a period of 22 minutes. The force of contractions then gradually increased until it became as great as it was before. There was however no pain.

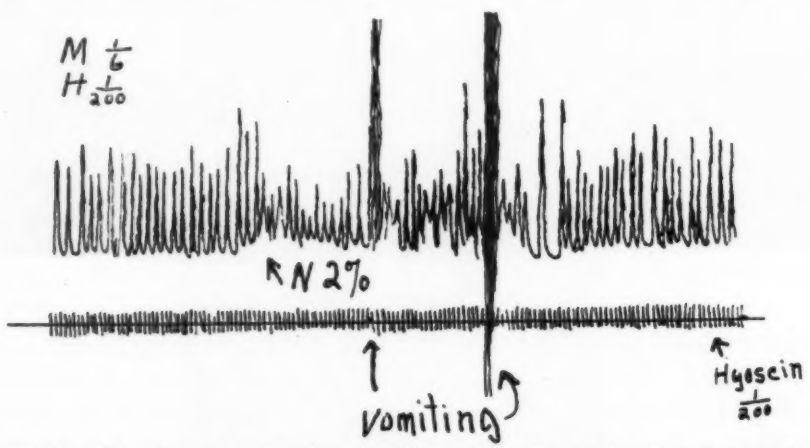

Fig. 11.—Internal hysterogram showing cessation of uterine contractions for an hour and fifteen minutes after sacral anesthesia with novocaine and adrenalin.

and of about one-half of their former height. This effect lasted for thirty-two minutes when the patient had a long hard contraction.

Novocaine given sacrally, has inconstant effects upon uterine contractions. According to Bonar and Meeker,[13] in the majority of their cases there was complete cessation of contractions within ten minutes after the injection was completed. "This diminution rarely lasted more than twenty minutes, after which the contractions gradually increased in frequency and duration until shortly the contractions proceeded normally." The majority of our

cases have conformed to this type and Fig. 10 is an excellent example. Here the contractions ceased in ten minutes and did not reappear for twenty-two minutes. In ten minutes more the contractions had regained their former force, but their duration was still less than it was before the novocaine was administered. The contractions were not accompanied by the sensation of pain. In some cases, however, there is a complete cessation of contractions as long as the effect of the novocaine lasts. Fig. 11 shows such a result. In only one of my cases was there an increase in the frequency of the contractions after the use of novocaine in this manner. In this patient (Fig. 12) the pains immediately increased and were fully as strong as formerly. Had I not obtained perfect relaxation and anesthesia, I would have thought

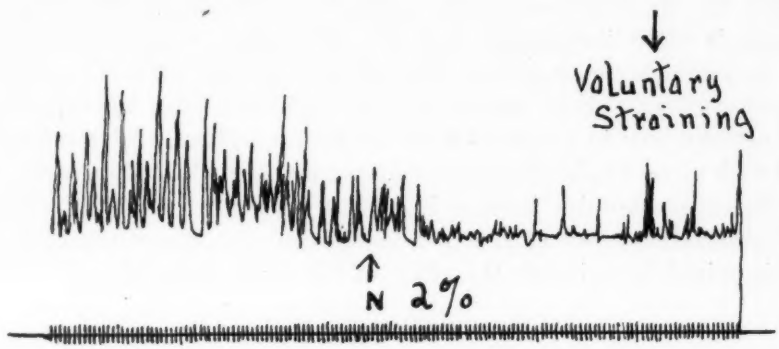

Fig. 12.—Internal hysterogram showing an unusual effect of novocaine given sacrally. Here the contractions were more frequent and were fully as strong. This patient was a primipara and was delivered by version. She had excellent relaxation and anesthesia.

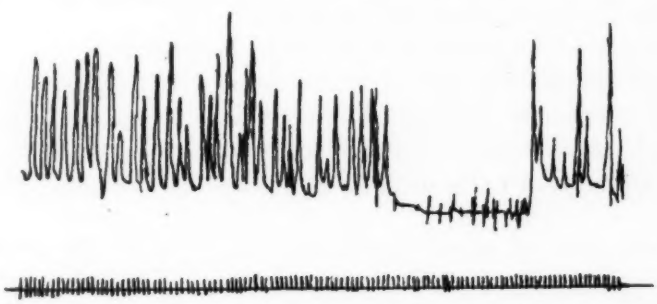

Fig. 13.—Internal hysterogram showing effect of puncture without giving any drug. In this case I was unable to locate the sacral hiatus with my needle. Nevertheless the contractions ceased for 28 minutes.

that I failed to give the novocaine into the sacral canal and was getting only an adrenal effect. The patient was extremely neurotic and I purposely did not give her any drugs of any kind. I simply applied a 5 per cent cocaine solution in 1-1000 acriflavine to the vulva and to the cervix before placing the bag, and when she reached the latter part of the first stage, I gave her novocaine sacrally. There was no sensation of pain after the novocaine was injected. She was delivered by version and there was extreme relaxation of the perineum and cervix.

Fig. 13 shows the effect that may follow a puncture in the region of the sacral hiatus. In this case, I was unable to get my needle into the hiatus

and therefore did not give any novocaine except the few drops that were used to anesthetize the skin preparatory to the puncture with the spinal needle. This hysterogram shows graphically the well-known phychic control of uterine contractions. The inhibition lasted twenty-eight minutes.

SUMMARY

It is important that the obstetrician know of an anesthetic, not only its safety in regard to both mother and child, but also the effect upon uterine contractions, in order that he may choose intelligently the proper anesthetic for any given case. The effect of an anesthetic upon the uterus not only influences the progress of labor, but also the ease of intrauterine manipulations, and the likelihood of atonic postpartum bleeding. The behavior of the uterus is often inconstant, and this fact must constantly be borne in mind. It would seem, however, that all anesthetics with the possible exception of ethylene, have a tendency to diminish the force and frequency of uterine contractions in proportion to the amount given. This effect is less marked with nitrous oxide-oxygen, and most marked with chloroform. Sacral anesthesia with novocaine belongs in a class by itself. Its usual effect is to stop all contractions soon after it is administered, but the contractions return with unimpaired force before the effect of the drug wears off.

CONCLUSIONS

The patient who has a Voorhees bag within her cervix offers an excellent opportunity of studying the effect of the appropriate anesthetic in her case upon the contractions of her uterus.

The behavior of the uterus is remarkably inconstant.

Inhalation anesthetics have a tendency to diminish both the force and frequency of uterine contractions in proportion to depth of the anesthesia. This action is most marked with chloroform, almost as great with ether, much less with nitrous oxide-oxygen, and scarcely, if at all, noticeable with ethylene.

The usual effect of sacral anesthesia with novocaine is to stop all contractions for a variable period of from twenty to fifty-five minutes. The contractions then reappear with gradually increasing force until they reach their former level, but are not accompanied by pain. Sacral anesthesia may however stop all contractions until the action of the drug wears off, or it may diminish the force of contractions without interfering with their rhythm, or, in very exceptional cases, it may increase the rhythm without changing their force.

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LABORATORY METHODS

UREA DETERMINATIONS IN BLOOD AND URINE*

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THE methods for the determination of urea in urine and in blood which are given in this paper have been gradually developed by a number of workers in this laboratory during the past ten years. They are based on the procedures published by Marshall[1] in 1913. Some modifications were described by Addis and Watanabe[2] in 1916. Since then several important alterations have been made but for the last two or three years there has been no essential change. They are now more accurate and considerably more convenient than before and they may be useful to those who are interested in the study of Bright's disease because they are particularly designed to meet the conditions met with in clinical investigation. But it should be remembered that they are recommended only for the determination of urea in urine and in whole blood kept from coagulating by potassium oxalate or sodium fluorid. In spite of a great deal of investigation we have been unable as yet to explain the discrepancies which are found when parallel determinations on whole blood, plasma and blood and plasma filtrates are compared. As a matter of fact very little is known in regard to the urease method. The mechanism of the decomposition of urea by urease is not yet decided[3] and even such a fundamental matter as the structure of the urea molecule is still uncertain.[4] It is well to recognize, therefore, that all urease methods are more or less empirical and that accordingly they demand a close observance of those apparently arbitrary requirements which experience has shown to be necessary.

SOLUTIONS

1. *Urease.*—This solution is freshly prepared each day. Whole jack beans are ground to a fine powder in an "Arcade" mill. The beans should not be more than one year old.† Ten grams of the powder is shaken up in 100 c.c. of water and incubated at 37° C. for thirty minutes. The filtrate is called urease.

2. *Inactivated Urease.*—Five c.c. of 1 per cent mercuric chloride solution is mixed with 45 c.c. of urease.

3. *Buffer Mixture.*—Eighteen liters of this solution are made at one time from concentrated stock solutions. One hundred and eighty c.c. of a solu-

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†As far as I can ascertain there is only one farmer in the United States who has a crop of jack beans every year (Mr. G. C. Reeves, Mineola, Texas). The beans are of no value except as a source of urease, and the demand is so small that there may be some danger of the supply failing altogether. We have not succeeded in growing them here. They apparently require a hot climate.

tion containing 1.4 per cent sodium pyrophosphate and 0.2 per cent glacial phosphoric acid, and ninety c.c. of 0.1 N NaOH, are measured out in graduates and washed into an ordinary 19-liter distilled water bottle. Distilled water is run in, forty-five c.c. of 0.2 per cent rosolic acid dissolved in 70 per cent alcohol are added, and the solution is brought to the 18-liter mark and mixed. Toluol may be added to prevent the growth of moulds.

4. *Standard Urea Solution.*—A good grade urea is dissolved to saturation in hot alcohol. After cooling, the urea crystals are collected on a filter, redissolved and recrystallized as before, filtered and dried. A solution containing 0.3 per cent urea in water is prepared. It should be remembered that this solution may undergo bacterial decomposition even in the presence of toluol, but urea can be kept indefinitely in solution if it is dissolved in 0.01 N HCl. It is best, therefore, to make a 3 per cent solution in 0.01 N HCl, and to prepare a fresh 0.3 per cent solution by diluting 100 c.c. of this solution and 100 c.c. 0.01 N NaOH to 1000 c.c. with water.

5. *Barium Hydrate.*—The theoretical amount of barium hydrate required to make 18 liters of 0.01 N, is weighed and transferred to a 19-liter bottle. Boiled distilled water is added to the 18-liter mark. The exact normality is determined by titration with potassium acid phthalate as described by Dodge.[5] The phthalate can now be obtained in a very pure form for use as a standard in electrometric determinations of H-ion concentration.

6. *Hydrochloric Acid.*—An approximately 0.1 N solution is made by transferring the theoretical volume of concentrated hydrochloric acid from a graduate into a 19-liter bottle and filling to the 18-liter mark with distilled water. The concentration of the concentrated hydrochloric acid used can be read off in a table[6] from the specific gravity given on the label. The actual normality of the solution obtained in this manner is then carefully determined by repeated titrations of heated samples with a known solution of barium hydrate, using phenolphthalein as an indicator. No attempt is made to get an exactly 0.1 N solution.

7. *Saturated Potassium Carbonate.*—The C. P. brands of potassium carbonate often contain ammonia and are expensive. We buy U. S. P. potassium carbonate in 100 lb. lots.* It is sometimes so dirty that it has to be filtered but we have never found ammonia. Saturated solutions are made in a series of vessels which are not so large that they cannot be easily handled and the contents are filtered into an 18-liter distilled water bottle. It is convenient to provide this large stock bottle with a syphon arrangement, and to draw off enough for each day's work into a small bottle.

THE METHOD FOR URINE

The optimal conditions are met when the urine contains from 0.3 per cent to 0.9 per cent urea. Urine obtained after diuresis from urea and water can be used directly, but specimens obtained in tests designed to test the urea concentrating capacity of the kidney should be diluted three to five times with water.

*Powers Weightman Rosengarten Co.

One hundred c.c. of the buffer mixture is run from an automatic measuring device into each of three 250 c.c. capacity Erlenmeyer flasks. Five c.c. of urine is pipetted into each flask. If the contents of the flasks are not still pink after the addition of the urine the P_H is so far to the acid side that the activity of the urease may be inhibited, and 0.1 N NaOH is then added in equal amount to each flask until the pink color returns. To the first flask, 2 c.c. of inactivated urease is now added; to the second, 2 c.c. of urease and 5 c.c. of 0.3 per cent urea; and to the third, 2 c.c. of urease.

The flasks are allowed to stand at room temperature for forty-five minutes. In this laboratory the temperature varies very little from 20° C. but in hot climates a shorter time might be desirable, for after more than two hours at 20° C. there may be a slight loss of ammonia by diffusion from the open flasks.

At the end of the period of incubation two drops of 0.5 per cent methyl orange are added. The first flask is then titrated with the known hydrochloric acid solution (approximately 0.1 N). As the contents become neutral, the pink color due to the presence of rosolic acid disappears; as more acid is added the solution becomes cloudy on account of the precipitation of a protein in the urease and still later a pink tinge appears which is due to the methyl orange. The particular shade of pink taken as the end-point is immaterial but whatever is chosen becomes the end-point for the two other flasks which are titrated to the same tinge of color. The titration value of the urease alone is obtained by adding 2 c.c. to a flask containing 100 c.c. of water with methyl orange and rosolic acid which has been brought to the pink color chosen for the end-point, and the amount of acid to bring back this color is noted. The same procedure is used to find the value of the inactivated urease.

When 3 mg. of urea are completely decomposed the ammonia produced neutralizes 1 c.c. of 0.1 N acid. The second and third flasks were alike except that 15 mg. of urea (5 c.c. of 0.3 per cent urea solution) were added to the second. The second flask should therefore require 5 c.c. of 0.1 N acid more than the third. If it does, we are assured of the adequacy of the urease and also obtain a duplicate determination of the urea in the urine. When the titration figure for the inactive urease is subtracted from the number of c.c. of acid required for the first flask, we obtain the alkali value of urine in which no ammonia has been produced from urea. This urine value plus the urease value when substracted from the value for the third flask, gives the number of c.c. of acid neutralized by ammonia derived from the urea in 5 c.c. of urine. If the acid used is exactly 0.1 N the number of c.c. multiplied by three will give the amount of urea in 5 c.c. of urine in milligrams. The actual calculations are, however, carried out with the help of a slide rule because the acid is practically never exactly 0.1 N and errors are apt to be made when several figures have to be multiplied. The amount of urea in the whole specimen of urine is then obtained by multiplying by the volume in c.c. divided by five.

THE METHOD FOR BLOOD

The end of the piston of a dry 5 c.c. syringe is smeared with vaseline and touched to finely powdered potassium oxalate or sodium fluorid so that a little sticks to the vaseline. The needles are sterilized in paraffin oil. About 5 c.c. of blood is drawn from a vein, the needle is detached, the piston removed, the nozzle closed with the forefinger of the left hand and 2 c.c. of the blood pipetted directly from the barrel into a "blood tube." We calibrate bulb pipettes to contain, not to deliver, for the viscosity of blood may vary so much that the delivery amounts are inconstant. The pipettes must therefore be washed free from all traces of blood. For this purpose a little porcelain crucible holding 6 c.c. is filled with a solution prepared by diluting 20 c.c. of a mixture of 1.4 per cent sodium pyrophosphate and 0.2 per cent glacial phosphoric acid to 1000 c.c. with water, and this 6 c.c. amount is used for the washing of the pipette. Another 2 c.c. volume of blood is then washed into another tube, and two other tubes are prepared to which only the washing fluid is added. Two c.c. of the urease used in the urine method are pipetted into each of the four tubes, the contents are mixed by shaking, the rubber corks are inserted and they are allowed to stand at room temperature for an hour.

Equal amounts (about 10 c.c.) of approximately 0.02 N hydrochloric acid, to which just enough methyl red (1 per cent solution in 70 per cent alcohol) has been added to give it a deep red color, are now placed in four acid tubes and boiled distilled water is run in until the level of fluid is 6 cm. high. When the period of incubation is ended, the blood and acid tubes are connected in series to a source of compressed air for aeration. The air passes first through a tube which contains 5 per cent sulphuric acid in order that it may be freed from all traces of ammonia, and is carried through the first blood tube to the first acid tube, and then to the second blood and acid tubes. Duplicate determinations are always run in tandem, because occasionally it is possible to demonstrate that a trace of ammonia escapes the acid in the first tube and is caught in the second. Since we may assume that this very small amount of ammonia is also lost from the second tube the titration of this tube will be more nearly correct and the first is used as a check. After all the connections have been made, the stoppers of the tubes containing the blood and urease or the urease alone are momentarily opened to allow of the introduction of a few drops of caprylic alcohol and of 16 c.c. of a saturated solution of potassium carbonate which is run in from a rapid delivery pipette. The air is gradually turned on and after a slow stream has passed through the tubes for five minutes the second acid tube in each series is connected with a water manometer which measures the rate of air flow. The valves are turned until a rate of five and a half liters per minute is attained. Thirty minutes aeration at five and a half liters per minute is required. The titrations are carried out directly in the acid tubes using approximately 0.01 N barium hydrate.

The difference between the titration value of the tubes containing urease and the tubes containing blood as well as urease gives the alkali equivalent of the ammonia produced from the decomposition of the urea in the blood.

One c.c. of 0.01 N barium hydrate is equivalent to 0.3 mg. of urea, so the number of c.c. of 0.01 N barium hydrate multiplied by 0.3 and by 50 will give the number of mg. of urea in 100 c.c. of blood.

In some laboratories there may be difficulty in securing a sufficiently constant air pressure. We had no compressed air supply available during the first few years, and after finding that water suction, no matter what apparatus was used, was inadequate, we filled a large tank with compressed air by means of a pump. But the needle valves which controlled the flow of air into the tubes had to be regulated at frequent intervals in order to maintain an approximately constant rate of flow. Even when a compressed air system was introduced, the varying use in other parts of the building induced too much fluctuation in the pressure in the laboratory. An entirely satisfactory and constant supply was only obtained when a pressure of 40 lbs. to the square inch was maintained by an automatically acting pump in a very large tank. This pressure is relayed to a large hot water tank in the laboratory and from it to two batteries each with eight needle valves. Even when all the sixteen outflows are being used the pressure remains constant and free from any measurable fluctuation.

APPARATUS

1. *Glassware.*—In this laboratory, urea determinations in six to twelve short time urine collections often have to be made, and we have found it best to have the subjects void urine in the laboratory directly into rubber stoppered bottles of about 1000 c.c. capacity. The name and the time over which the specimen was collected is written on the bottle with a grease pencil and it is placed in chronological series with the others on a table on which rows of Erlenmeyer flasks containing the buffer solution have been arranged three deep. If these positions are maintained it is not necessary to number the flasks. Very small amounts of mercury or of any heavy metal depress the activity of urease, so the flasks in the first row, into which the inactivated urease is pipetted, are permanently marked with black paint, and the flasks in which urea is decomposed are never used for any other titration. It is convenient to have these bottles and flasks set on a table which runs on rubber wheels so that when the urine volumes are measured or diluted or the flasks emptied and washed the table may be pushed up to the sink. When the work is done and the flasks have been rinsed with tap water they are set in the inverted position in holes bored in the top of the table.

The tubes used in the blood method are made from pyrex tubing and are sixteen and one-fourth inches long and have an internal diameter of one and one-eighth inches. They are closed with a No. 6 rubber stopper, through which pass two tubes of 4 mm. internal diameter, one running to the bottom of the tube and the other a short bent tube for the exit of the air during aeration. With these long tubes and with the volumes of fluid we deal with, there is no need of any device to break up the bubbles of air, nor is it necessary to employ any mechanism to prevent the carrying over of fluid from one tube to another.

The tubes are carried in wooden frames each of which holds four sets

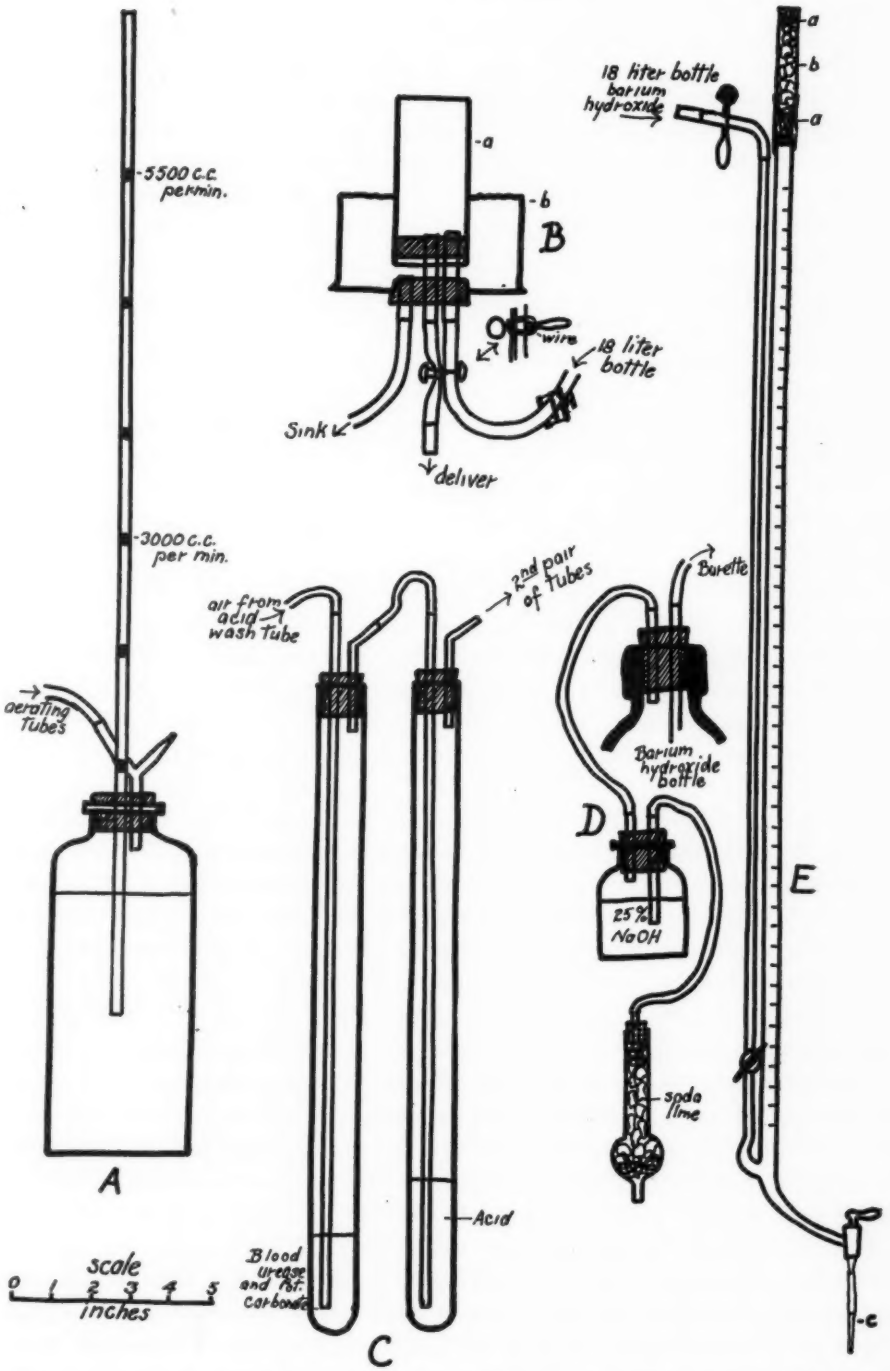

Fig. 1.—*A*, Meter for determining rate of air flow during aeration. *B*, Automatic device for rapidly delivering 100 c.c. of buffer solution: *a*, large glass tube; *b*, bottom of a large tin can. *C*, tubes set up for aeration, preceded by a tube of acid for washing the air (not drawn) and followed by tubes for blood and for acid. *D*, Means of protecting the barium hydroxide from carbon dioxide. *E*, Self-filling burette used for barium hydroxide; *a*, glass wool; *b*, soda lime; *c*, flexible tip. A similar burette without the carbon dioxide protection is used for the acid in the urine urea determination.

of three. They are conveniently set on a low wooden table which runs on wheels. The blood tubes are thoroughly rinsed with tap water after they have been used, and when not in use are always kept full of water. The neutral solution obtained at the completion of the titration with barium hydrate is left standing in the acid tubes until they are used again, when the fluid is run out and the tube is rinsed with boiled distilled water. When the tubes are new a little alkali may come from the glass, but in much-used tubes the yellowing of the neutral fluid which is left in them is due to the fading of the indicator.

2. *Self-filling Burettes.*—A great deal of time is saved by a device such as is shown in Fig. 1, whereby burettes can be rapidly filled. It is essential for the barium hydrate solution which, of course, would rapidly grow weaker if exposed to air containing carbon dioxide. With an adequate protection by soda lime tubes the strength may remain unaltered for weeks, though it should be checked at intervals by titration against acid potassium phthalate.

3. *Automatic Measuring Device for the Buffer Solution.*—This is very easily made and saves time in filling the flasks. In Fig. 1 the mechanism is drawn to scale.

4. *Measurment of Rate of Flow of Air.*—This is simply a bottle of about 1000 c.c. capacity half full of water with a long tube running to the bottom through a rubber cork and a narrow tube for the egress of air. The height to which the water rises in the tube is proportional to the rate of air flow. The tube is calibrated by noting with a stop-watch the time required to displace water from a vessel of known volume inverted in water.

THE COLLECTION OF URINE AND OF BLOOD

In the collection of urine there is always a danger that the bladder will not be completely emptied even though the subject is asked to make the collection as complete as possible. The possibility that urine will be left in the bladder is considerable when the volume collected is less than 100 c.c., and diminishes as the volume increases until with 500 c.c. volumes there is likely to be very little error if the cooperation of the subject is obtained. When approximately hourly collections are being made, the production of a diuresis is, therefore, one of the necessary elements for the attainment of accuracy. In certain patients the volume of urine per hour will not exceed 100 c.c. even when large amounts of water have been taken, and under these circumstances it is better to collect urine at one and a half or at two hour intervals. The collections are made in a room which opens off the laboratory. A commode with a glass funnel emptying into a 1000 c.c. bottle is used for the women, while the men collect the urine directly in the bottles. The timing is made at the moment when the collection is completed, not when it is begun, and the subjects are usually asked to note this time and to deliver the collection immediately in the laboratory where their memorandum is checked. Hourly urine collections are made in series so that an error due to incomplete voiding of urine may often be suspected when a marked irregularity in the successive amounts of urea is found. The urine estimations are made within an hour or two of collection but if for any reason this is impossible a sufficient

amount of 5 per cent sulphuric acid should be added to make the specimen strongly acid. This is the only effective method we have found to prevent the bacterial decomposition of urea.

Blood is taken at the middle of the short time periods of urine collection, and if that is not done the successive blood urea concentration values are plotted on coordinate paper and the average blood urea concentration for each period is calculated from the mean of the ordinates.

There are few exceptions to the general rule that it is a waste of time to work with urine and blood which has been collected by nurses or internes. Their other duties and their general training unfit them for accuracy in timing.

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SOME OF THE MORE PRACTICAL METHODS OF EXAMINATION OF THE BLOOD IN TUBERCULOSIS*

By Henry C. Sweany, M.D., Chicago, Ill.

CONSIDERING the importance of the disease, there is too little information gained from a blood examination in tuberculosis. Owing to the fact that the blood offers a most important means for the study of diseases, it seems that a greater effort should be exerted in this field to assist the clinician in diagnosis, prognosis and treatment of our most universal and until recently, most prevalent disease. With this aim in mind, we have been doing extensive work in our laboratory on blood chemistry and to a limited extent along other allied lines. We wish, therefore, to bring to date the various reactions that have been serviceable in the past and add the results of more recent findings that we feel will be of use; discussing these topics for convenience under the headings immunologic, cytologic, and chemical.

No doubt the greatest possibilities exist in this field of immunology, although at the present time they have not become evident. There were many encouraging reports a decade ago concerning complement fixation in tuberculosis. Besredka, Craig, Miller, Inman, Bronfenbrenner and others reported flattering results. Petroff in his reports is a trifle more conservative. The work of Corper and Sweany and others at that time resulted in a more pessimistic outlook. After eight years I can say that my opinion has changed little. Over ten thousand tests have been performed in our institution, using

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Corper's Antigen with more or less hit and miss results. Two thousand tests were tabulated and studied but there was noted no advantage over our previous reports so the work was not published.

At present, we are using the new Wassermann antigen which seems to possess many advantages over any other method. Wassermann works on the hypothesis that there are antibodies developed for both lipoids and proteins and therefore he combines a tetralin extract of the tubercle bacillus with nonspecific lecithin. More positive results are obtained with this antigen than we have obtained with any other antigen, although there exist certain discrepancies which will be reported on later from this laboratory. The fact is evident that by mixing an anticomplementary lipoid extract with an anticomplementary lecithin in certain proportions an antigen is obtained that possesses a "range" of greater dimension. This does not occur with either lipoid or protein extract alone. After a trial of seventeen different antigens, specific and nonspecific extracts of the tubercle bacillus and other bacilli none of them were found to possess antigenic value except where mixtures existed such as are present in Corper's or Besredka's antigen. The pure extracts possessed almost as much anticomplementary power as they did antigenic strength. Just why a "range" should occur on mixing we have no way of explaining unless it is as a result of binding complement. It may be that by more accurate and refined technic with more specific antigens we may yet attain the long-sought method for early diagnosis along these lines, but until then the complement-fixation reaction in tuberculosis must be evaluated with a great deal of caution. The positive tests may not always be specific and the negative tests may occur in almost any stage of the active disease—more especially in the later stages. Before the reaction can become reliable the anticomplementary substances must be reduced to insignificant traces, otherwise the reaction will be due in part to anticomplementary factors and the remainder a combination of specific or nonspecific factors produced by a complex of substances that are adsorbed to the complementary aggregate and prevent it from acting.

Precipitin and agglutinin reactions have never given results worthy of confidence. Our results with the precipitin test on two hundred cases were approximately of equal value to those obtained with the complement-fixation reaction although by no means did they run parallel. The precipitin reaction was performed by making a ring test in a small tube by superimposing Corper's antigen over the serum and reading the reaction after two hours' residence of the tubes in a Uhlenhuth rack.

More recently the Wildbolz reaction gave promise of value but this test has also been shown to have many inherent faults so that it is doubtful whether these will be eventually overcome.

Thus we leave the field of humoral antibodies in tuberculosis with practically nothing dependable accomplished in so far as laboratory reactions of practical value are concerned.

Turning now to cytologic examinations, there are certain of these that merit consideration if properly performed and carefully interpreted. The Arneth nuclear classification indicates the trend of the disease but is by no

means constant enough to afford material aid. Likewise a red, white and differential count on the blood gives a limited amount of information. Only when performed and interpreted as we propose to show, is there sufficient information gained to warrant doing them. In Table I are listed the examinations in eight patients all having about the same quantity of massive involvement but varying in type of the process. The first patient is what may be classed as a healed fibroid and each one succeeding in order is of a more exudative state. The last patient died three days after the examinations were made. The Group V patients are fibroids that have healed or are healing; Group II are stationary and Group I are the exudative types or proliferative types that have become exudative. Of the patients that lived, most have been observed for several years.

Beginning in the first column it is noticeable that the percentage of red cells gradually decreases from about normal, 45 per cent, to 30 per cent, a decrease of about 30 per cent. Presumably the converse is true if the patient recovers. The red cell count, however, changes only about 10 to 15 per cent. By combining these two values the volume index described by Capps[1] is obtained where the actual volume of each red cell is obtained expressed in cubic microns. This result is more significant. The volume of the red cell decreases from a normal of $90\mu^3$ to about $68\mu^3$ in the terminal stage. By multiplying these two results and dividing by 100 an exaggerated figure is obtained which may be termed the efficiency index. Those cases that fall below 30 are failing or not doing well. These results may be obtained by means of a red cell count and an hematocrite reading. The latter must be performed in a high speed instrument for eight to ten minutes at 6000 to 8000 r.p.m. or one hour at 2500 r.p.m.

The hemoglobin and color index are of some value but they are not as constant as the "efficiency index."

The white blood cells fluctuate in various ways and in general are not altogether satisfactory. The "lymphocytic index" is perhaps of most value and offers a fair gauge of the patient's condition. The total disappearance of eosinophiles is a sign of bad prognosis. The Arneth count has been mentioned before. The total white cell count is not consistent. The "transitionals" and the large mononuclears, however, are of definite significance and may be used to indicate the progress of the patient. If the large mononuclears are less than 1 per cent, with the transitionals less than 6 per cent, the condition is satisfactory or good; if the large mononuclears increase to 2 to 6 per cent the condition is less favorable; if in addition, the transitionals increase to 8 to 12 per cent the condition is unsatisfactory; and just before death both disappear from the blood. We can agree with Cunningham[2] and others in the origin of the transitional cell. In certain cases of advanced tuberculosis there is a good demonstration of this fact. It seems that when a patient suddenly experiences an acute exacerbation there is a sudden switch from the large mononuclears to the transitionals. During this time a complete series has been repeatedly observed from the large mononuclear through to the fully developed "transitional" or "endothelial leucocyte" of Mallory, showing that

TABLE I

CYTOLOGIC EXAMINATIONS OF THE BLOOD OF VARIOUS TYPES OF FAR ADVANCED TUBERCULOSIS

NAME	GROUP	% CELLS	R. B. C. PER M. M.	HB.	COLOR INDEX	VOL. INDEX	EFFICIENCY INDEX	W. B. C. PER M.	POLYS.	LYM.	EOS.	L. MON.	TRANS.	ARNETH
J.O'B.	V	45.6	4.85	94.0	1.00	91.0	41.5	6.8	71.0	25.0	2.0	0	2.0	45.6
									[72.0	20.0	5.0	0	3.0	60.0]*
T.H.	V	41.5	4.70	75.0	0.89	88.0	41.0	9.3	57.0	26.0	1.0	2	3.0	
R.M.	V	39.0	4.52	75.0	0.92	86.0	34.4	10.2	79.0	21.0	1.0			
J.G.	II	34.6	4.26	51.0	0.69	81.0	28.0	15.0	73.0	11.5	2.0	6.5	8.0	71.9
F.E.	II	35.0	5.77	85.0	0.82	64.0	22.4	12.1	64.0	22.9	0	3.3	9.4	71.0
J.Dad	I	36.8	5.04	69.0	0.77	73.0	24.8	11.4	79.0	7.7	1.0	2.0	11.3	75.0
J.Dog	I	28.0	4.23	37.0	0.49	66.0	18.5	22.4	78.0	7.5	0	4.8	10.5	74.0
A.O.	I	30.7	4.56	68.0	0.83	67.0	20.7	11.5	78.0	14.0	0	0	3.0	85.0

the stimulation has become an irritation and has caused an increased number of the irritation forms of the cell.

While there are valuable clues relative to the patient's condition obtainable from immunologic and cytologic data, it is my opinion that ultimately a chemical examination of the blood will furnish the most information. Quite recently we[3] carried on chemical studies on the blood of tuberculosis individuals with certain interesting findings, the most important of which was the "unbalance" of blood lipoids. In tuberculosis, as with certain other diseases, the toxemia appears to throw the various lipoids out of normal equilibrium. This unbalanced condition prevails during the various vicissitudes of the disease and does not come to normal until a complete recovery ensues. As this condition can only occur in mild tuberculosis it means that the lipoids remain out of normal balance during a great part of the time in far advanced tuberculosis. We found that cholesterol, and to a less extent lecithin and fats, fluctuated above and below normal, approximately in proportion to the proliferation or exudation present, and that a rising or falling lipoid value was a fair index of the patient's condition.

Continuing this work in a study of phosphorous compounds McCluskey[4] has shown that the lecithin and acid-soluble phosphorus play a reciprocal rôle. As the lecithin rises and falls the acid-soluble phosphorus fluctuates in the opposite direction. There is never any great change in the total phosphorus but the reciprocal relation of the two groups of ingredients furnishes valuable information relative to the patient's condition. She showed that the first changes took place in the plasma followed by a change in the cells. In other words the cells act as a storehouse from which the plasma draws when in need and stores up when there is an excess.

Still more recently Sweany[5] has studied the cholesterol and cholesterol ester partition between cells and plasma. Of these compounds the fluctuation between cells and plasma is very similar to that of phosphorus, but the esters show the greatest fluctuation and appear to be the most valuable index of any of the blood ingredients thus far studied. They certainly furnish the most valuable information from the clinical laboratory standpoint, because the greatest changes occur in the plasma (in fact, there are practically no esters found in the cells) and the changes are so marked that they are more readily interpreted. The total cholesterol in the whole blood varied from the low figure of 115 mg. per 100 c.c. of blood in the exudative types to over 300 mg. in the proliferative. The total cholesterol in the cellular portion of the blood ranged from 63.0 to 116 mg., while in the plasma portion the figures ranged from 51.0 to 253 mg. The cholesterol in the cells is almost all free, leaving the plasma to contain most of the esters. The free cholesterol in the plasma ranged from 29.0 mg. to 142 mg. but this constituent in plasma is inconstant. One arrested patient had practically no free cholesterol in the plasma. The esters, however, were more constant, ranging from 22.0 mg. to 176 mg. This latter ingredient in the plasma, therefore, appears to be of far greater significance than any of the others. If the values are calculated on 100 c.c. of plasma the results appear far more exaggerated, giving values that range

between 33 and 300 mg. The ratios between the extreme types of the disease are greatest when calculated on this basis.

Any information relative to the explanation of these changes is lacking. We merely know that they take place in tuberculosis. Luden[6] has reported lipoid changes in cancer. Perhaps every disease affects the balance of the lipoids to a more or less degree. But so far as tuberculosis is concerned, there are definite changes that take place concomitant with the condition of the patient. Whether the changes are a result of toxemia causing a change of cell permeability or colloid stability, a defensive mechanism, or simply the result of the presence of pus, we are still unable to answer.

We attempted to get some light on the latter mentioned point by analyzing sputum in different stages of the disease. We found that the cholesterol content is proportionate to the leucocytes, that it is approximately the same in the leucocytes as in the blood of the individual and that there were practically no esters present. It is conceivable that a patient loses the cholesterol (and lecithin which is present in about 1 per cent concentration) by expectorating a liter of sputum a day. This would be equal to about 1 gram of cholesterol and 10 grams of lecithin. On a low lipoid diet in advanced tuberculosis it is quite possible that the lipoids are thus depleted. The changes in the proliferative types are not so readily accounted for on this basis, because there is not a constant accumulation of pus to give the increase. It is true there is a gradual diminution of sputum and the system may be so adapted to the "forced draft" of lipoid metabolism that it goes on producing them at an increased rate until a gradual readjustment takes place. Neither do lipoids parallel a leucocytosis. In the patient that is not expectorating, yet where there is enough toxemia to produce an excess of leucocytes, there may be (but not necessarily) a parallel lipoid change. The diet cannot be responsible for it because the patients that gave such high results were on the same general diet as those that gave lower results. Although the source of lipoids is more exogenous rather than endogenous, yet the actual level is regulated by conditions within, possibly by the endocrines as suggested by Luden.

As the patient begins to approach normal the cholesterol is possibly changed to esters and oxidized or prepared for elimination; thus do we have the changes taking place that we have cited above.

SUMMARY

1. A review of the more practical laboratory procedures in blood examination in tuberculosis is given. There is nothing reliable in a diagnostic way, although definite prognostic aid may be obtained by certain carefully performed examinations.

2. In the immunologic field there is little to look forward to at present. Complement fixation must still be considered in the experimental stage. It only offers indefinite aid prognostically.

3. In the cytologic field the percentage of red blood cells, taken with the individual cell volume (volume index), the "lymphocytic index," the eosinophile count and an accurate study of the endothelial type of cells (large

mononuclears and transitionals) are all points that may be used in prognostication.

4. Of the blood chemistry reactions, the lecithin, acid-soluble phosphorus, cholesterol and cholesterol ester determinations on the plasma furnish the simplest and most practical blood chemical methods, and ones which we have found to be of definite prognostic aid.

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(*For discussion, see p.* 422.)

ODD LABORATORY APPLIANCES*

BY FRANCIS LOWELL BURNETT, M.D., BOSTON, MASS.

THE work of a clinical laboratory generally consists in the application of instruments of precision to the determination of the tissues, injurious agents, or forces of the body. In most instances articles on this subject deal with the results obtained from the use of instruments and appliances, and this of course is the most valuable part of scientific investigation. But the apparatus used may in some measure add to the accuracy and facility with which the results are obtained, and therefore I wish to illustrate and describe a few odd laboratory appliances with the hope that some of you may find them useful.

THE LABORATORY BENCH

(1) The laboratory bench contains a few new features. It extends along two sides of a room in a level and continuous line with the soapstone sinks and draining boards as shown in Fig. 1. The top of the bench is twenty-two inches wide and thirty-two inches above the linoleum. A powerful centrifugal machine is conveniently placed in the corner of the bench and the top projects a little above its surface. Along the front is a facing five inches wide and set back an inch from the outer edge of the bench. The front of several drawers forms part of this facing, but above them and immediately below the bench is a gas pipe (1). On this line of pipe there are numerous cocks (1) which control burners at the back of the bench. Then there are also electric switches (2) that operate outlets, the centrifugal machine and the daylite lamp. In a few convenient places there are rings

*Read before the Third Annual Meeting of the American Society of Clinical Pathologists, Rochester, Minn., June 5-7, 1924.

to which safety pins are attached (2). A large pin is used for holding a towel; and a small one by piercing the edge of several sizes of filter paper keeps them in an orderly and handy position. The gas outlets by means of an elbow have a horizontal attachment for rubber tubing or else are attached directly to a curved Bunsen burner (3) which pierces a soapstone gutter at the back of the bench.

THE GUTTER

This gutter is six inches across and four inches in depth. A galvanized 1-inch L iron is bolted to the back to support rods and asbestos mats for

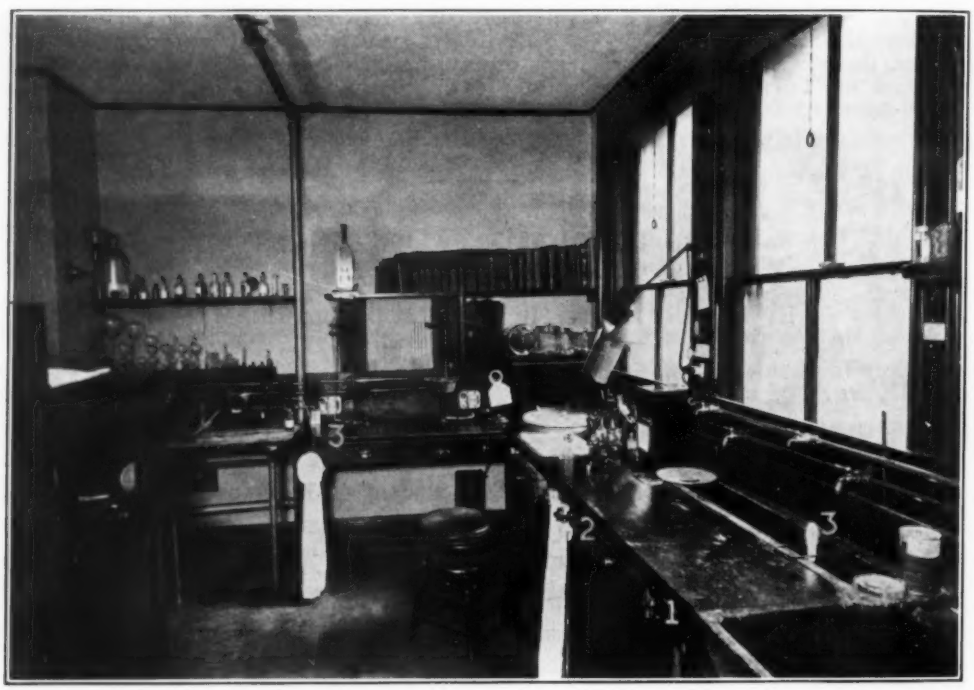

Fig. 1.—A general view of laboratory bench showing the gutter, the daylite lamp, the top of centrifugal machine (Corner), the gas and electric controls (2) on facing, and the gas outlets (3).

heating flasks and pans of fluid. The main purpose of the gutter, however, is to catch the drip from steaming stains and wash water from the small bibcocks above. The gutter has a drain that can be plugged. On a seat in front of part of the bench a worker can operate various burners, or electrical devices, and can stain, steam or wash smears without moving.

SOAKING BASIN

At each end of the bench there is a soapstone draining board and sink. The larger sink is divided by a partition, which encloses a soaking basin of 20 x 12 x 5 in. This has its own hot and cold water cocks, and also a plug for the drainpipe. This basin forms a convenient receptacle for soaking dirty and used glassware.

DRYING RACK

Immediately above each sink is a drying rack, Fig. 2, (4), which is made on the principle of a milk-can horse commonly seen outside the farm houses in New England. In this instance however it is made with a concavo-convex surface with short pegs, one, two and three inches long and one-fourth, three-eighths and one-half inches in diameter. It may be made of any length or width, although a projection of five inches from the wall is sufficient not to interfere with other appliances. The pegs, too, may be placed at any distance from one another according to the glassware to be dried or stored. Such a device not only furnishes a convenient rack for

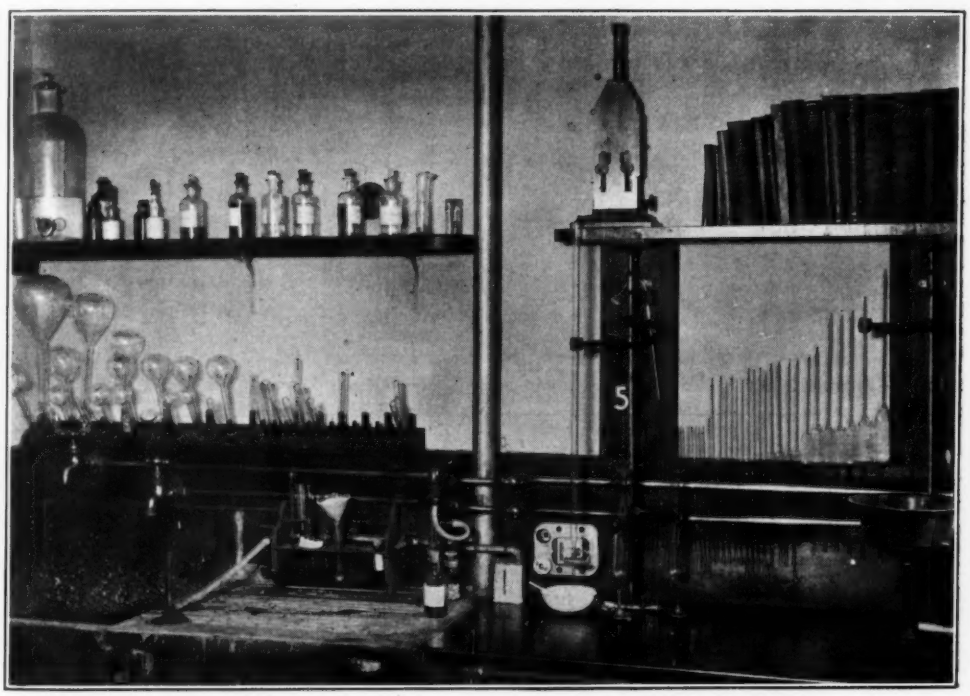

Fig. 2.—A close view of the bench showing the drying rack (4), the fixed ring and clamp uprights (5), and the volumetric pipette holder.

drying, but also a very compact and convenient means of keeping clean bottles, volumetric flasks and tubes. The volumetric pipettes are also very conveniently kept in order at the back of the water pipes.

CLAMP AND RING UPRIGHT

The metal support to which rings, clamps, etc., are attached, is a cumbersome piece of apparatus with a clumsy and heavy base that often takes up a great deal of room on a bench. To obviate the difficulties encountered with a movable stand, fixed uprights—Fig. 2, (5)—of one-eighth of an inch galvanized iron pipe are attached to the surface of the bench at one end and support a bookshelf at the other. Such a permanent and fixed upright is not only convenient and useful, but it furnishes an unusually steady sup-

port for rings holding pans of fluid, and also clamps for large tubes and burette.

DAYLITE LAMP

Since the invention of daylite glass several lamps have been put on the market for the illumination of laboratory apparatus. Most of them are small and adapted only for lighting a microscope. With a large lamp having an

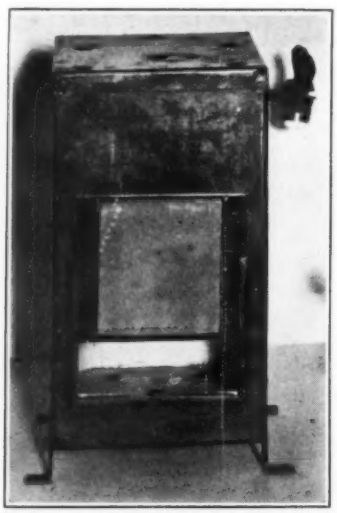

Fig. 3.—A daylite glass lamp to use for a microscope, colorimeter or dark-field illumination.

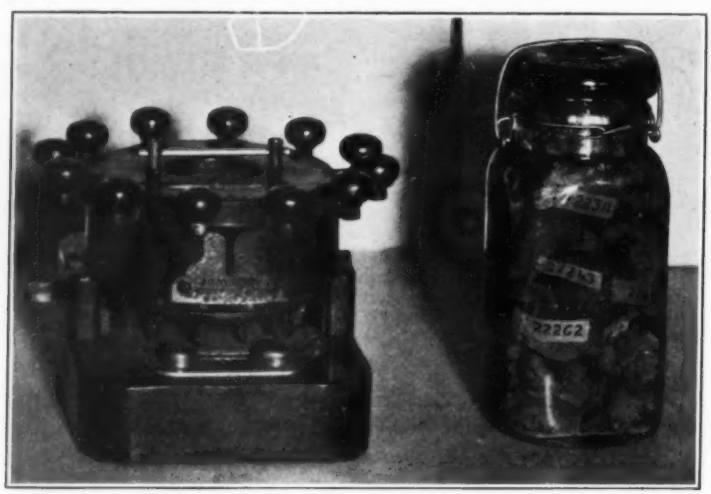

Fig. 4.—A perforating machine to number tags for tissue, and a jar containing some of the fixed, preserved and numbered specimens.

illuminating area of sixteen square inches as shown in Fig. 3, a constant and good light can be had at all times for the microscope and colorimeter. Then, too, the daylite glass window can be raised and the very intense light of a 200-watt nitrogen filled bulb used for a steady and good dark-field illumination. The body of the lamp case measures 10 × 7 × 6 in. outside and is supported by four legs an inch high. In the permanent floor and the movable

top is a double layer of metal with three-fourths inch holes. The six holes in the layers of the top and bottom are not apposed, so that while the inside of the lamp is well ventilated the rays of light do not project outside. Across the interior there is a metal bar which supports the electric light socket from which wires run to a plug on the outside. The inside of the case is silvered and there is a reflector at the back of the lamp.

NUMBERING AND PRESERVING TISSUE

In many laboratories, especially those in which research and teaching are carried on, there are shelves and shelves of numbered or labelled bottles containing pieces of tissue. After the tissue is put into the bottle it is not often wanted again. On the other hand if the pieces of tissue are pierced with a thread and tied to a label of tough paper which is numbered by a check per-

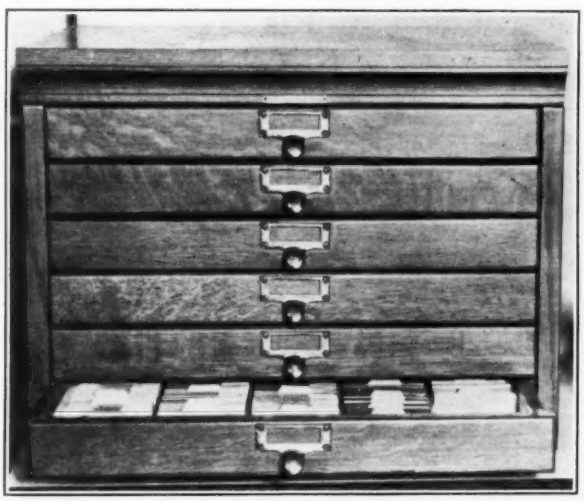

Fig. 5.—Cabinet for mounted and stained sections in which each drawer contains 750, and the case 4,500 slides.

forating machine (Fig. 4), all of the specimens can be put into a large receptacle. If the pieces of tissue are too small or friable to be strung on a thread, they may be put into a piece of gauze which is bound up with a thread piercing the label. In this way a great many bottles and corks and a large amount of alcohol will be saved. A quart jar filled with many numbered specimens is shown in Fig. 4.

A CABINET FOR MOUNTED SLIDES

Stained sections of tissue are also conveniently stored in a case of drawers illustrated in Fig. 5. This cabinet is of a standard make of the demi unit type,* and on this account innumerable sections may be obtained and placed one on top of another. Each drawer is divided by four partitions which form five rows in which the slides are nicely fitted. One hundred and fifty slides of medium thickness and some index cards can be kept in each row. With

*Library Bureau Legal Blank Demi Unit No. 9726.

five rows each drawer will hold 750 slides, and with six drawers each cabinet will hold 4,500 slides.

CULTURE TUBES

Bacteriology developed out of chemistry and still contains a few relics of the past. One of these is the long test tube with a cotton plug for culture media. In Fig. 6 are shown some culture tubes of agar-agar, blood agar, blood serum, and bouillon. The tubes instead of being five or six inches long are only three inches long and three-fourths inch wide inside diameter. This length of tube allows sufficient room for a good slant. They also have a square base and will stand upright; and instead of the customary cotton plug a cork stopper is used. To sterilize the tube, a groove is cut longitudinally in the small end of the cork and is inserted a short distance. The groove allows the steam an opportunity to reach the inside of the tube, and yet soon

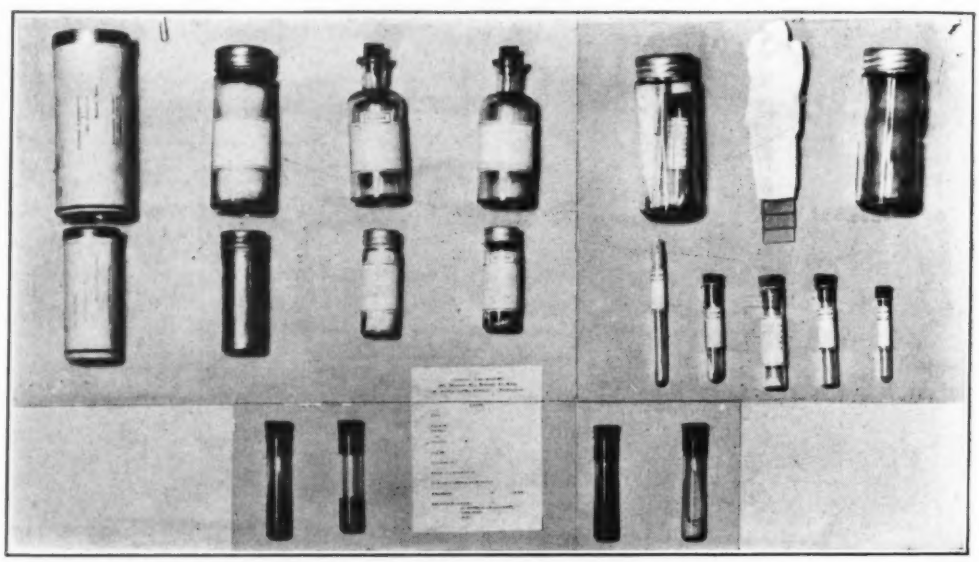

Fig. 6.—The two upper rows show mailing cases, bottles, jars, etc., for specimens. The lower row illustrates short, square bottomed and corked tubes of media.

after sterilizing, the corks are soft and plastic and can be inserted further and the tube kept air tight.

There are many advantages of this kind of tube for media. In the first place it is economical in saving glass, and it saves time in making a plug; corks can be used several times. In the second place it prevents the evaporation of the water of condensation, drying of solid media, or a fluid media from running out if the tube tips over. In the third place it will stand upright without a rack. And lastly, on account of its short length, it greatly facilitates touching individual colonies.

JARS OF MEDIA

Jars* of 120 c.c. and 30 c.c. capacity with a screw cap and rubber washer are also conveniently used for larger amounts of media. To sterilize, the cap

*Round screw cap jar. Whitall Tatum Co.

is unscrewed a little, and then made tight when this process has been accomplished. The surface of solid media in the larger jar is equal to that of the ordinary Petri dish and the colonies are almost as accessible. Fluid media is kept hermetically sealed.

AN ASSORTMENT OF GLASSWARE FOR SPECIMENS

In Fig. 6 is shown a jar of 120 c.c. capacity which holds most of the glassware immediately about it, that is to say, it contains a 10 c.c. tube with oxylate, a 5 c.c. sterile tube, a culture tube, a 2 c.c. sterile tube containing a hollow needle, and three slides wrapped in a sponge. This assortment of glassware, which only occupies a space of $4\frac{1}{2} \times 2$ in., may be carried conveniently by consultants and practitioners and almost any or all specimens obtained from a patient.

SWAB TUBES

The two tubes on the left of lower row in Fig. 6 illustrate two swabs. The outer is 4 inches long and $\frac{1}{4}$ inch in diameter and holds a maple stick with a swab of cotton on the lower end. At the upper end the stick is wrapped with a piece of cotton tape to increase the diameter and make a good fit for a short piece of rubber tubing which in turn fits the glass tube.

Another form of swab is shown in the next tube. This is made of No. 14 copper wire and nickel plated. One end has a one-eighth inch loop and a swab at the other. Then it is bent in the middle, put into the tube, a grooved cork inserted, and the whole sterilized. For long swabs the wire is easily straightened, but swabs that cannot be transferred to media immediately will remain moist for some time in this tight tube.

The other illustrations consist of a 30 c.c. sterile jar, and one containing a piece of gauze soaked in formalin for tissue. Then there is a 120 c.c. jar of the same kind, 90 c.c. bottles, and a tin case used for a jar in mailing infectious material.

MAILING CASES AND WRAPPERS

Any of the glassware fits easily into one of the mailing cases. The case has a wrapper of thin tough paper which is gummed along one edge, and is held at the bottom by several turns of a rubber band which fits well into a groove. The top of the wrapper is held by the screw cap. In sending the case by mail a label with two gummed and perforated edges is addressed to a doctor or hospital and pasted over the address of the laboratory on the wrapper. Then when the case is returned it is merely necessary to tear off the label between the perforations and the case is addressed to the laboratory.

The odd laboratory appliances illustrated and described in this article are so far as I know original. At any rate they are not in general use. Accordingly if you have to consider the practical side of laboratory work you may find some of these devices and appliances valuable.

TRANSACTIONS

THIRD ANNUAL CONVENTION AMERICAN SOCIETY OF CLINICAL PATHOLOGISTS, ROCHESTER, MINNESOTA

June 5-7, 1924

(Concluded.)

DR. J. H. BLACK, DALLAS, TEXAS, read a paper entitled **Pollen Sensitization: A Preliminary Report.** (For original article, see page 378.)

DR. O. J. WEST, SEATTLE, WASH., read a paper entitled **Is Pathologic Nomenclature Either Scientific or Sensible?** (For original article, see page 384.)

DR. FRANCIS LOWELL BURNETT, BOSTON, MASS., read a paper entitled **Odd Laboratory Appliances.** (For original article, see page 415.)

DR. HENRY C. SWEANY, CHICAGO, read a paper entitled **Some of the More Practical Methods of Examination of the Blood in Tuberculosis.** (For original article, see page 409.)

Doctor Corper.—I have not very much to add to Dr. Sweany's presentation, except that I should like to point out the significance of this contribution in tuberculosis, a point which I believe was not emphasized in the presentation.

This paper has at least one important significance aside from the practical points dwelt upon by Dr. Sweany. For the past fourteen years Dr. H. G. Wells and his colleagues have been interested in the chemistry of tuberculosis and as a culmination of their studies and a review of reports in the literature have contributed recently the first and a most excellent text volume on this phase of medicine. The information on blood chemistry had to be taken from many different sources and as such was patched information and not entirely satisfactory, many studies having been made with obsolete and inaccurate methods. Dr. Sweany and his colleagues have presented to us a complete study of the blood by the most modern and approved methods for blood chemistry. From this standpoint, you can readily appreciate that the work is not only of practical, but also of definite original scientific value.

I wish to compliment Dr. Sweany and his colleagues for this contribution to our knowledge in tuberculosis.

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EDITORIALS

The Hospital Laboratory: Its Scope, Function and Relation to Hospital Efficiency

MODERN studies in hospital efficiency may be summed up in the somewhat obvious conclusion that hospital efficiency is fundamentally dependent upon the efficiency, not of any special departments, but upon the efficiency of the hospital as a whole.

The functions of the modern hospital, regardless of size, are multiple and indivisible and closely linked with the general efficiency of the hospital as a whole; all are of equal importance and none capable of undue accentuation at the expense of the others. They embody much more than the housing of the sick. They may be looked upon as: (1) The scientific, skilful, and ethical care of the sick; (2) Teaching, as concerns the nurse, the interne, the staff, and the community; and (3) Research, as concerned with the evaluation of old methods, and the evolution of new—whether concerned with the diagno-

sis or treatment or with the better understanding of the mechanism and manifestations of disease.

The function of the hospital laboratory in the practical realization of these objectives is, or should be, obvious. The scientific care of the sick; intelligent and effective teaching; the acquisition and dissemination of knowledge. Productive studies and constructive research do not and cannot result from the accumulation or analysis of isolated facts.

Diagnosis, the *sine qua non* of intelligent and scientific treatment, teaching and research, depend, not upon the accumulation of a mass of isolated and perhaps not obviously related facts, but essentially upon their interpretation and application to the particular problem; upon the correlation of one fact with another.

The scientific, careful, or even the intelligent study of the problems confronting the hospital in the fulfilment of its duties or functions is impossible without the active cooperation of the laboratory. That every case, without exception, entering the hospital should be subjected to certain fundamental laboratory studies is now generally admitted; many will demand further and more extensive investigations, but none can escape or fail to utilize the absolute minimum. The laboratory, however, which confines its work solely to the collection and issuance of a mass of statistical data fails entirely in its true function, for the laboratory and the wards, the laboratory and the hospital, cannot be separated successfully.

The laboratory, through its studies, may bring forth and establish certain facts as suggested by the presence or absence of certain findings, but the laboratory, through its director, should also have an active part in the correlation of these facts with the others also elicited in the particular case; unless it is in liaison with all the other departments engaged in the study of a particular problem, its full duty and its highest efficiency fail of accomplishment.

Proper, complete, and active liaison between the laboratory and the clinical departments of the hospital is essential as, also, is the recognition of the fact that, specialized as the practice of medicine has become, there is no specialty so distinct as to enable it to withdraw from contact with medicine as a whole; the proper understanding of one necessitates, at least, an intelligent conception of the others.

Without a properly functioning laboratory, a properly functioning hospital is impossible and the proper functioning of the laboratory depends, not upon the number of reports issued, but upon the degree to which it is concerned with, and utilized in the study of disease and the interpretation and analysis of its manifestations.

It is plain that this conception necessitates the entrance of the laboratory into the wards, as it were, and its active and consulting participation in clinical problems.

As it has been stated by Stillman:[1] "What should be the attitude of the laboratory toward requests for examinations made by the clinical division? Should it merely perform the examinations and say nothing * * * or should it intrude itself into the wards, urge the adoption of some methods

and the dropping of others, and generally cooperate in the attempt to solve the clinician's problems? Obviously, the latter position is the only one tenable if the laboratory is to be of the greatest possible service in every respect." If the former only is accepted, then "all that is necessary is the installation of sufficient technicians to do the work."

The modern clinician realizes that the laboratory and its personnel constitute a highly specialized department of the hospital and that the hospital comprises a mechanism composed of highly specialized units working in coordinated harmony. The efficiency of the hospital laboratory is mainly influenced by and dependent upon the ability and efficiency of its director, who should be chosen, not merely for his manipulative efficiency in various technical laboratory procedures, but for his ability to supervise the work of his assistants, to eliminate lost motion, and, above all, for his perspective, vision, and ability to coordinate and correlate the work of his department with the work of the clinical divisions.

The director must, therefore, have had a well-grounded training in the clinical arts in order that he may be able to interpret in terms of the patient the pathology or abnormalities he demonstrates in the laboratory—which, in essence, constitutes the difference between the clinical pathologist and the technician, however competent or expert the latter in technical manipulations.

The College of Surgeons has recognized these necessities in including among the minimum hospital requirements that a clinical laboratory in charge of a clinical pathologist must be "available"—but there is still, in places, a hiatus between the availability and the utilization of these requirements.

Every hospital will have its own problems, each laboratory its particular difficulties; the solution of the problem lies in a recognition of the fact that laboratory and clinical medicine are indissolubly linked together; that the laboratory is—or should be—a highly specialized department of clinical medicine; that "the pathologist is—or should be—a well-trained clinician and diagnostician equally well trained, in addition, in the performance and interpretation of laboratory investigations * * * a consultant from the standpoint of *what* to do as well as how to do it,"[2] and that it is the duty of the clinician to be sufficiently acquainted with the advances in ways and means of laboratory investigations to use them to the best advantage.

REFERENCES

[1]Stillman, R. G.: A Criticism of Hospital Laboratory Examinations, Jour. Amer. Med. Assn., June 25, 1921, lxxvi, 1816.

[2]Kilduffe, R. A.: Function of the Pathologist as a Consultant, Jour. Amer. Med. Assn., January 1, 1921, lxxvi, 54.

—*R. A. K.*

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The Next Annual Meeting Will Be Held in Philadelphia May 21-23, 1925
Prepare for the Coming Convention

A letter and questionnaire have been sent out to all our members apprising them of our next annual meeting which is to be held May 21, 22 and 23, 1925, in Philadelphia, the home city of our President, Dr. Kolmer. Under his inspiration the gathering next year bids fair to excel our previous successful conventions in scientific contributions to our specialty and advancement of our cause. A new and useful feature will be the commercial exhibit of instruments, apparatus and reagents for the laboratory worker. Members should make plans now for attending our next meeting. Dr. Burdick will be pleased to make hotel reservations not only for the Philadelphia meeting of the A. S. C. P. but he is also in a position to extend the service to include reservations in Atlantic City for the A. M. A. convention which is held the week following ours. Those who have papers to present, and we hope there will be many, will communicate at once with the secretary, Dr. Ward Burdick, 652 Metropolitan Bldg., Denver, Colorado